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(54) Title: RECOMBINANT IL-18 ANTAGONISTS USEFUL IN TREATMENT OF IL-18 MEDIATED DISORDERS			
(57) Abstract <p>Chimeric, humanized and other IL-18 mAbs, derived from high affinity neutralizing mAbs, pharmaceutical compositions containing same, methods of treatment and diagnostics are provided.</p>			

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RECOMBINANT IL-18 ANTAGONISTS USEFUL IN TREATMENT OF IL-18
MEDIATED DISORDERS

5 FIELD OF THE INVENTION

The present invention relates generally to the field of antibodies and altered antibodies, useful in the treatment and diagnosis of conditions mediated by IL-18, and more specifically to mAbs, Fabs, chimeric and humanized antibodies.

10 BACKGROUND OF THE INVENTION

Human interleukin-18 is a recently identified cytokine that is synthesized as a biologically inactive 193 amino acid precursor protein (Ushio et al., *J. Immunol.* 156:4274, 1996). Cleavage of the precursor protein, for example by caspase-1 or caspase-4, liberates the 156 amino acid mature protein (Gu et al., *Science* 275:206, 15 1997; Ghayur et al., *Nature* 386:619, 1997), which exhibits biological activities that include the costimulation of T cell proliferation, the enhancement of NK cell cytotoxicity, the induction of IFN- γ production by T cells and NK cells, and the potentiation of T helper type 1 (Th1) differentiation (Okamura et al., *Nature* 378:88, 1995; Ushio et al., *J. Immunol.* 156:4274, 1996; Micallef et al., *Eur. J. Immunol.* 20 26:1647, 1996; Kohno et al., *J. Immunol.* 158:1541, 1997; Zhang et al., *Infect. Immunol.* 65:3594, 1997; Robinson et al., *Immunity* 7:571, 1997). In addition, IL-18 is an efficacious inducer of human monocyte proinflammatory mediators, including IL-8, tumor necrosis factor- α (TNF- α), and prostaglandin E₂ (PGE₂) (Ushio, S. et al., *J. Immunol.* 156:4274-4279, 1996; Pure, A.J. et al., *J. Clin. Invest.* 25 10:711-721, 1997; Podolin et al., *J. Immunol.* submitted, 1999).

The previously cloned IL-1 receptor-related protein (IL-1Rrp) (Parnet et al., *J. Biol. Chem.* 271:3967, 1996) was recently identified as a subunit of the IL-18 receptor ($K_d = 18$ nM) (Torigoe et al., *J. Biol. Chem.* 272:25737, 1997). A second subunit of the IL-18 receptor exhibits homology to the IL-1 receptor accessory protein, and has been termed AcPL (for accessory protein-like). Expression of both IL-1Rrp and AcPL are required for IL-18-induced NF- κ B and JNK activation (Born et al., *J. Biol. Chem.* 273:29445, 1998). In addition to NF- κ B and JNK, IL-18 signals through IL-1 receptor-associated kinase (IRAK), p56lck (LCK), and mitogen-activated protein kinase (MAPK) (Micallef et al., *Eur. J. Immunol.* 30 26:1647, 1996; Matsumoto et al., *Biophys Biochem. Res. Comm.* 234:454, 1997; Tsuji-Takayama et al., *Biochem. Biophys. Res. Comm.* 237:126, 1997).

Th1 cells, which produce proinflammatory cytokines such as IFN- γ , IL-2 and TNF- β (Mosmann et al., J. Immunol. 136:2348, 1986), have been implicated in mediating many of autoimmune diseases, including multiple sclerosis (MS), rheumatoid arthritis (RA), type 1, or insulin dependent, diabetes (IDDM). 5 inflammatory bowel disease (IBD), and psoriasis (Mosmann and Sad, Immunol. Today 17:138, 1996). Thus, antagonism of a Th1-promoting cytokine such as IL-18 would be expected to inhibit disease development. IL-18 specific mAbs could be used as an antagonist.

10 The role of IL-18 in the development of autoimmune diseases has been demonstrated. Accordingly, it has been demonstrated that IL-18 expression is significantly increased in the pancreas and spleen of the nonobese diabetic (NOD) mouse immediately prior to the onset of disease (Rothe et al., J. Clin. Invest. 99:469, 1997). Similarly, IL-18 levels have been shown to be markedly elevated in the synovial fluid of rheumatoid arthritis patients (Kawashima et al., Arthritis and 15 Rheumatism 39:598, 1996). Furthermore, it has been demonstrated that IL-18 administration increases the clinical severity of murine experimental allergic encephalomyelitis (EAE), a Th1-mediated autoimmune disease that is a model for multiple sclerosis. In addition, it has been shown that neutralizing anti-rat IL-18 antiserum prevents the development of EAE in female Lewis rats (Wildbaum et al., J. Immunol. 161:6368, 1998). Accordingly, IL-18 is a desirable target for the 20 development of a novel therapeutic for autoimmunity.

25 Taniguchi et al., J. Immunol. Methods 206:107, describe seven murine and six rat anti-human IL-18 mAbs, which bind to four distinct antigenic sites. One of the murine mAbs (#125-2H), and the six rat mAbs inhibit IL-18-induced IFN- γ production by KG-1 cells, with the rat mAbs exhibiting neutralizing activities 10-fold lower than that of #125-2H. As demonstrated by Western blot analysis, three of the murine mAbs, but none of the rat mAbs, are strongly reactive with membrane-bound human IL-18. In addition, an enzyme-linked immunosorbent assay (ELISA) to detect human IL-18 is described, utilizing #125-2H and a rat mAb. The limit of 30 detection of this ELISA is 10 pg/ml.

35 European patent application EP 0 712 931 discloses two mouse anti-human IL-18 mAbs, H1 (IgG1) and H2 (IgM). As demonstrated by Western blot analysis, both mAbs react with membrane-bound human IL-18, but not with membrane-bound human IL-12. H1 is utilized in an immunoaffinity chromatography protocol to purify human IL-18, and in an ELISA to measure human IL-18. H2 is utilized in a radioimmunoassay to measure human IL-18.

Neutralizing IL-18 antibodies may potentially be useful in relieving autoimmune diseases and related symptoms in man. Hence there is a need in the art for a high affinity IL-18 antagonist, such as a neutralizing monoclonal antibody to human interleukin 18, which would reduce Th1 differentiation and proliferation and thus autoimmune diseases and related symptoms.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides rodent (e.g., rat and murine) neutralizing monoclonal antibodies specific for human interleukin-18 and having a binding affinity characterized by a dissociation constant equal to or less than about 3.9×10^{-11} M as described in the detailed description. Exemplary of such monoclonal antibodies are the rat monoclonal antibody 2C10 and rat and murine monoclonal antibodies such as 14B7 and 13G9. Another aspect of the invention are hybridomas such as 19522C10(2)F2(1)A1, 195214B7(1)H10 and 187413G9(3)F12.

In a related aspect, the present invention provides neutralizing Fab fragments or $F(ab')_2$ fragments thereof specific for human interleukin-18 produced by deleting the Fc region of the rodent neutralizing monoclonal antibodies of the present invention.

In still another related aspect, the present invention provides an altered antibody specific for human interleukin-18 which comprises complementarity determining regions (CDRs) derived from a non-human neutralizing monoclonal antibody (mAb) characterized by a dissociation constant equal to or less than about 3.9×10^{-11} M for human interleukin-18 and nucleic acid molecules encoding the same. When the altered antibody is a humanized antibody, the sequences that encode complementarity determining regions (CDRs) from a non-human immunoglobulin are inserted into a first immunoglobulin partner in which at least one, and preferably all complementarity determining regions (CDRs) of the first immunoglobulin partner are replaced by CDRs from the non-human monoclonal antibody. Preferably, the first immunoglobulin partner is operatively linked to a second immunoglobulin partner as well, which comprises all or a part of an immunoglobulin constant chain.

In a related aspect, the present invention provides CDRs derived from non-human neutralizing monoclonal antibodies (mAbs) characterized by a dissociation constant equal to or less than about 3.9×10^{-11} M for human interleukin-18, and nucleic acid molecules encoding such CDRs.

In still another aspect, there is provided a chimeric antibody containing human heavy and light chain constant regions and heavy and light chain variable

regions derived from non-human neutralizing monoclonal antibodies characterized by a dissociation constant equal to or less than about 3.9×10^{-11} M for human interleukin-18.

5 In yet another aspect, the present invention provides a pharmaceutical composition which contains one (or more) of the above described altered antibodies and a pharmaceutically acceptable carrier.

10 In a further aspect, the present invention provides a method for treating conditions in humans associated with excess Th1 production, for example autoimmune diseases, by administering to said human an effective amount of the pharmaceutical composition of the invention.

15 In yet another aspect, the present invention provides methods for, and components useful in, the recombinant production of altered antibodies (e.g., engineered antibodies, CDRs, Fab or $F(ab)_2$ fragments, or analogs thereof) which are derived from non-human neutralizing monoclonal antibodies (mAbs) characterized by a dissociation constant equal to or less than 3.9×10^{-11} M for human IL-18. These components include isolated nucleic acid sequences encoding same, recombinant plasmids containing the nucleic acid sequences under the control of selected regulatory sequences which are capable of directing the expression thereof in host cells (preferably mammalian) transfected with the recombinant plasmids.

20 The production method involves culturing a transfected host cell line of the present invention under conditions such that an altered antibody, preferably a humanized antibody, is expressed in said cells and isolating the expressed product therefrom.

25 In yet another aspect of the invention is a method to diagnose conditions associated with excess Th1 production in a human which comprises obtaining a sample of biological fluid from a patient and allowing the antibodies and altered antibodies of the instant invention to come in contact with such sample under conditions such that an IL-18/antibody (monoclonal or altered) complex is formed and detecting the presence or absence of said IL-18/antibody complex.

30 Other aspects and advantages of the present invention are described further in the detailed description and the preferred embodiments thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

35 Fig. 1 [SEQ ID NOS: 1 and 2] illustrates the light chain variable region for the rat antibody 2C10. Fig. 1 includes sequence data for both strands. The boxed areas indicate the CDR's [SEQ ID NOS: 3-8]. The bolded area indicates the degenerate primer sequence.

Fig. 2. [SEQ ID NOS: 9 and 10] illustrates the heavy chain variable region for the rat antibody 2C10. Fig. 2 includes sequence data for both strands. The boxed areas indicate the CDR's [SEQ ID NOS: 11-16]. The bolded area indicates the degenerate primer sequence.

5 Fig. 3 [SEQ ID NOS: 17 and 18] illustrates the light chain variable region for the murine antibody 13G9. Fig. 3 includes sequence data for both strands. The boxed areas indicate the CDR's [SEQ ID NOS: 19-24]. The bolded area indicates the degenerate primer sequence.

10 Fig 4 [SEQ ID NOS: 25 and 26] illustrates the heavy chain variable region for the murine antibody 13G9. Fig. 4 includes sequence data for both strands. The boxed areas indicate the CDR's [SEQ ID NOS: 27-32]. The bolded area indicates the degenerate primer sequence.

15 Fig 5 [SEQ ID NOS: 33 and 34] illustrates the light chain variable region for the rat antibody 14B7. Fig. 5 includes sequence data for both strands. The boxed areas indicate the CDR's [SEQ ID NOS: 35-40]. The bolded area indicates the degenerate primer sequence.

20 Fig 6 [SEQ ID NOS: 41 and 42] illustrates the heavy chain variable region for the rat antibody 14B7. Fig. 6 includes sequence data for both strands. The boxed areas indicate the CDR's [SEQ ID NOS: 43-48]. The bolded area indicates the degenerate primer sequence.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a variety of antibodies, altered antibodies and fragments thereof, which are characterized by human IL-18 binding specificity, 25 neutralizing activity, and high affinity for human IL-18 as exemplified in rat monoclonal antibody 2C10, murine monoclonal antibody 13G9 and rat monoclonal antibody 14B7. The antibodies of the present invention were prepared by conventional hybridoma techniques to generate novel neutralizing antibodies. These products are useful in therapeutic and pharmaceutical compositions for treating IL- 30 18-mediated disorders, e.g. autoimmune diseases, including multiple sclerosis (MS), rheumatoid arthritis (RA), type 1, or insulin dependent, diabetes (IDDM), inflammatory bowel disease (IBD), and psoriasis (Mosmann and Sad, Immunol. Today 17:138, 1996). These products are also useful in the diagnosis of IL-18-mediated conditions by measurement (e.g., enzyme linked immunosorbent assay 35 (ELISA)) of endogenous IL-18 levels in humans or IL-18 released *ex vivo* from activated cells.

I. Definitions.

"Altered antibody" refers to a protein encoded by an altered immunoglobulin coding region, which may be obtained by expression in a selected host cell. Such altered antibodies are engineered antibodies (e.g., chimeric or humanized antibodies) or antibody fragments lacking all or part of an immunoglobulin constant region, e.g., 5 Fv, Fab, or F(ab)₂ and the like.

"Altered immunoglobulin coding region" refers to a nucleic acid sequence encoding altered antibody of the invention. When the altered antibody is a CDR-grafted or humanized antibody, the sequences that encode the complementarity 10 determining regions (CDRs) from a non-human immunoglobulin are inserted into a first immunoglobulin partner comprising human variable framework sequences. Optionally, the first immunoglobulin partner is operatively linked to a second immunoglobulin partner.

"First immunoglobulin partner" refers to a nucleic acid sequence encoding a 15 human framework or human immunoglobulin variable region in which the native (or naturally-occurring) CDR-encoding regions are replaced by the CDR-encoding regions of a donor antibody. The human variable region can be an immunoglobulin heavy chain, a light chain (or both chains), an analog or functional fragments thereof. Such CDR regions, located within the variable region of antibodies 20 (immunoglobulins) can be determined by known methods in the art. For example Kabat et al. (Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987)) disclose rules for locating CDRs. In addition, computer programs are known which are useful for identifying CDR regions/structures.

"Neutralizing" refers to an antibody that inhibits IL-18 activity by preventing 25 the binding of human IL-18 to its specific receptor or by inhibiting the signaling of IL-18 through its receptor, should binding occur. A mAb is neutralizing if it is 90% effective, preferably 95% effective and most preferably 100% effective in inhibiting IL-18 activity as measured in the IL-18 neutralization assay, see Example 1 and 30 Table I.

The term "high affinity" refers to an antibody having a binding affinity characterized by a K_d equal to or less than 3.9×10^{-11} M for human IL-18 as determined by optical biosensor analysis (see Example 2 and Table I).

By "binding specificity for human IL-18" is meant a higher affinity for 35 human IL-18 than murine, or other IL-18.

"Second immunoglobulin partner" refers to another nucleotide sequence encoding a protein or peptide to which the first immunoglobulin partner is fused in

frame or by means of an optional conventional linker sequence (i.e.. operatively linked). Preferably it is an immunoglobulin gene. The second immunoglobulin partner may include a nucleic acid sequence encoding the entire constant region for the same (i.e., homologous - the first and second altered antibodies are derived from the same source) or an additional (i.e., heterologous) antibody of interest. It may be an immunoglobulin heavy chain or light chain (or both chains as part of a single polypeptide). The second immunoglobulin partner is not limited to a particular immunoglobulin class or isotype. In addition, the second immunoglobulin partner may comprise part of an immunoglobulin constant region, such as found in a Fab, or 10 F(ab)₂ (i.e., a discrete part of an appropriate human constant region or framework region). Such second immunoglobulin partner may also comprise a sequence encoding an integral membrane protein exposed on the outer surface of a host cell, e.g., as part of a phage display library, or a sequence encoding a protein for analytical or diagnostic detection, e.g., horseradish peroxidase, β -galactosidase, etc.

15 The terms Fv, Fc, Fd, Fab, or F(ab)₂ are used with their standard meanings (see, e.g., Harlow et al., Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory, (1988)).

20 As used herein, an "engineered antibody" describes a type of altered antibody, i.e., a full-length synthetic antibody (e.g., a chimeric or humanized antibody as opposed to an antibody fragment) in which a portion of the light and/or 25 heavy chain variable domains of a selected acceptor antibody are replaced by analogous parts from one or more donor antibodies which have specificity for the selected epitope. For example, such molecules may include antibodies characterized by a humanized heavy chain associated with an unmodified light chain (or chimeric light chain), or vice versa. Engineered antibodies may also be characterized by alteration of the nucleic acid sequences encoding the acceptor antibody light and/or 30 heavy variable domain framework regions in order to retain donor antibody binding specificity. These antibodies can comprise replacement of one or more CDRs (preferably all) from the acceptor antibody with CDRs from a donor antibody described herein.

A "chimeric antibody" refers to a type of engineered antibody which contains naturally-occurring variable region (light chain and heavy chains) derived from a donor antibody in association with light and heavy chain constant regions derived from an acceptor antibody.

35 A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more)

human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity (see, e.g., Queen et al., Proc. Natl Acad Sci USA, 86:10029-10032 (1989). Hodgson et al., Bio/Technology, 9:421 (1991)).

5 The term "donor antibody" refers to an antibody (monoclonal, or recombinant) which contributes the nucleic acid sequences of its variable regions, CDRs, or other functional fragments or analogs thereof to a first immunoglobulin partner, so as to provide the altered immunoglobulin coding region and resulting expressed altered antibody with the antigenic specificity and neutralizing activity characteristic of the donor antibody. One donor antibody suitable for use in this
10 invention is a non-human neutralizing monoclonal antibody (i.e., rat) designated as 2C10. The antibody 2C10 is defined as a high affinity, human-IL-18 specific (i.e., does not recognize murine IL-18), neutralizing antibody of isotype IgG_{1,K} having the variable light chain DNA and amino acid sequences of SEQ ID NOs: 1 and 2 and respectively, the variable heavy chain DNA and amino acid sequences of SEQ ID
15 NOs: 9 and 10 on a suitable murine IgG constant region.

20 The term "acceptor antibody" refers to an antibody (monoclonal, or recombinant) heterologous to the donor antibody, which contributes all (or any portion, but preferably all) of the nucleic acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the first immunoglobulin partner. Preferably a human antibody is the acceptor antibody.

25 "CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. See, e.g., Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987). There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein refers to all three heavy chain CDRs, or all three light chain CDRs (or both all heavy and all light chain CDRs, if appropriate).

30 CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. CDRs of interest in this invention are derived from donor antibody variable heavy and light chain sequences, and include analogs of the naturally occurring CDRs, which analogs also share or retain the same antigen binding specificity and/or neutralizing ability as the donor antibody from which they
35 were derived.

By sharing the antigen binding specificity or neutralizing ability is meant, for example, that although mAb 2C10 may be characterized by a certain level of antigen

affinity, a CDR encoded by a nucleic acid sequence of 2C10 in an appropriate structural environment may have a lower, or higher affinity. It is expected that CDRs of 2C10 in such environments will nevertheless recognize the same epitope(s) as 2C10. Exemplary light chain CDRs of 2C10 include

5 SEQ ID NO: 3;
SEQ ID NO: 5;
SEQ ID NO: 7;
and exemplary heavy chain CDRs of 2C10 include
SEQ ID NO: 11;
10 SEQ ID NO: 13;
and SEQ ID NO: 15.

A "functional fragment" is a partial heavy or light chain variable sequence (e.g., minor deletions at the amino or carboxy terminus of the immunoglobulin variable region) which retains the same antigen binding specificity and/or 15 neutralizing ability as the antibody from which the fragment was derived.

An "analog" is an amino acid sequence modified by at least one amino acid, wherein said modification can be chemical or a substitution or a rearrangement of a few amino acids (i.e., no more than 10), which modification permits the amino acid sequence to retain the biological characteristics, e.g., antigen specificity and high 20 affinity, of the unmodified sequence. For example, (silent) mutations can be constructed, via substitutions, when certain endonuclease restriction sites are created within or surrounding CDR-encoding regions.

Analogs may also arise as allelic variations. An "allelic variation or modification" is an alteration in the nucleic acid sequence encoding the amino acid 25 or peptide sequences of the invention. Such variations or modifications may be due to degeneracy in the genetic code or may be deliberately engineered to provide desired characteristics. These variations or modifications may or may not result in alterations in any encoded amino acid sequence.

The term "effector agents" refers to non-protein carrier molecules to which 30 the altered antibodies, and/or natural or synthetic light or heavy chains of the donor antibody or other fragments of the donor antibody may be associated by conventional means. Such non-protein carriers can include conventional carriers used in the diagnostic field, e.g., polystyrene or other plastic beads, polysaccharides, e.g., as used in the BIACore [Pharmacia] system, or other non-protein substances 35 useful in the medical field and safe for administration to humans and animals. Other effector agents may include a macrocycle, for chelating a heavy metal atom, or

radioisotopes. Such effector agents may also be useful to increase the half-life of the altered antibodies, e.g., polyethylene glycol.

II. High Affinity IL-18 Monoclonal Antibodies

5 For use in constructing the antibodies, altered antibodies and fragments of this invention, a non-human species (for example, bovine, ovine, monkey, chicken, rodent (e.g., murine and rat), etc.) may be employed to generate a desirable immunoglobulin upon presentation with native human IL-18 or a peptide epitope therefrom. Conventional hybridoma techniques are employed to provide a
10 hybridoma cell line secreting a non-human mAb to IL-18. Such hybridomas are then screened for binding using IL-18 coated to 96-well plates, as described in the Examples section, or alternatively with biotinylated IL-18 bound to a streptavidin coated plate.

15 One exemplary, high affinity, neutralizing mAb of this instant invention is mAb 2C10, a rat antibody which can be used for the development of a chimeric or humanized antibody, described in more detail in examples below. The 2C10 mAb is characterized by an antigen binding specificity for human IL-18 of about K_d 3.9×10^{-11} M. This mAb is characterized by being isotype IgG_{1,K}.

20 Another desirable donor antibody is the murine mAb 13G9. This mAb is characterized by being isotype IgG_{1,K}. The mAb has a dissociation constant for IL-18 of about 12×10^{-9} M.

Yet, another desirable donor antibody is the rat mAb, 14B7. This mAb is characterized by having a dissociation constant for IL-18 about 1.5×10^{-10} M. 14B7 is also characterized by being isotype IgG_{1,K}.

25 This invention is not limited to the use of the 13G9, 2C10, 14B7, or their hypervariable (i.e., CDR) sequences. Any other appropriate high affinity IL-18 antibodies characterized by a dissociation constant equal or less than about 3.9×10^{-11} M for human IL-18 and corresponding anti-IL-18 CDRs may be substituted therefor. Wherever in the following description the donor antibody is identified as 13G9,
30 2C10, 14B7, this designation is made for illustration and simplicity of description only.

III. Antibody Fragments

The present invention also includes the use of Fab fragments or $F(ab')_2$ fragments derived from mAbs directed against human IL-18. These fragments are useful as agents protective *in vivo* against IL-18 and Th1-mediated conditions or *in vitro* as part of an IL-18 diagnostic. A Fab fragment contains the entire light chain and amino terminal portion of the heavy chain; and an $F(ab')_2$ fragment is the fragment formed by two Fab fragments bound by disulfide bonds. MAbs 13G9, 2C10, 14B7, and other similar high affinity, IL-18 binding antibodies, provide sources of Fab fragments and $F(ab')_2$ fragments which can be obtained by conventional means, e.g., cleavage of the mAb with the appropriate proteolytic enzymes, papain and/or pepsin, or by recombinant methods. These Fab and $F(ab')_2$ fragments are useful themselves as therapeutic, prophylactic or diagnostic agents, and as donors of sequences including the variable regions and CDR sequences useful in the formation of recombinant or humanized antibodies as described herein.

The Fab and $F(ab')_2$ fragments can be constructed via a combinatorial phage library (see, e.g., Winter et al., *Ann. Rev. Immunol.*, **12**:433-455 (1994)) or via immunoglobulin chain shuffling (see, e.g., Marks et al., *Bio/Technology*, **10**:779-783 (1992), which are both hereby incorporated by reference in their entirety) wherein the Fd or v_H immunoglobulin from a selected antibody (e.g., 13G9) is allowed to associate with a repertoire of light chain immunoglobulins, v_L (or v_K), to form novel Fabs. Conversely, the light chain immunoglobulin from a selected antibody may be allowed to associate with a repertoire of heavy chain immunoglobulins, v_H (or Fd), to form novel Fabs.

IV. Anti-IL-18 Amino Acid and Nucleotide Sequences of Interest

The mAb 2C10 or other antibodies described above may contribute sequences, such as variable heavy and/or light chain peptide sequences, framework sequences, CDR sequences, functional fragments, and analogs thereof, and the nucleic acid sequences encoding them, useful in designing and obtaining various altered antibodies which are characterized by the antigen binding specificity of the donor antibody.

As one example, the present invention provides variable light chain and variable heavy chain sequences from the IL-18 mAb 2C10 and sequences derived therefrom.

The nucleic acid sequences of this invention, or fragments thereof, encoding the variable light chain and heavy chain peptide sequences are also useful for mutagenic introduction of specific changes within the nucleic acid sequences

encoding the CDRs or framework regions, and for incorporation of the resulting modified or fusion nucleic acid sequence into a plasmid for expression..

Taking into account the degeneracy of the genetic code, various coding sequences may be constructed which encode the variable heavy and light chain 5 amino acid sequences, and CDR sequences of the invention as well as functional fragments and analogs thereof which share the antigen specificity of the donor antibody. The isolated nucleic acid sequences of this invention, or fragments thereof, encoding the variable chain peptide sequences or CDRs can be used to produce altered antibodies, e.g., chimeric or humanized antibodies, or other 10 engineered antibodies of this invention when operatively combined with a second immunoglobulin partner.

It should be noted that in addition to isolated nucleic acid sequences encoding portions of the altered antibody and antibodies described herein, other such nucleic acid sequences are encompassed by the present invention, such as those 15 complementary to the native CDR-encoding sequences or complementary to the modified human framework regions surrounding the CDR-encoding regions. Useful DNA sequences include those sequences which hybridize under stringent hybridization conditions [see, T. Maniatis et al, Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387 to 389] to the DNA 20 sequences. An example of one such stringent hybridization condition is hybridization at 4XSSC at 65°C, followed by a washing in 0.1XSSC at 65°C for an hour. Alternatively an exemplary stringent hybridization condition is in 50% formamide, 4XSSC at 42°C. Preferably, these hybridizing DNA sequences are at least about 18 nucleotides in length, i.e., about the size of a CDR.

25

V. Altered Immunoglobulin Molecules And Altered Antibodies

Altered immunoglobulin molecules can encode altered antibodies which include engineered antibodies such as chimeric antibodies and humanized antibodies. A desired altered immunoglobulin coding region contains CDR- 30 encoding regions that encode peptides having the antigen specificity of an IL-18 antibody, preferably a high affinity antibody such as provided by the present invention, inserted into a first immunoglobulin partner (a human framework or human immunoglobulin variable region).

Preferably, the first immunoglobulin partner is operatively linked to a second 35 immunoglobulin partner. The second immunoglobulin partner is defined above, and may include a sequence encoding a second antibody region of interest, for example an Fc region. Second immunoglobulin partners may also include sequences

encoding another immunoglobulin to which the light or heavy chain constant region is fused in frame or by means of a linker sequence. Engineered antibodies directed against functional fragments or analogs of IL-18 may be designed to elicit enhanced binding with the same antibody.

5 The second immunoglobulin partner may also be associated with effector agents as defined above, including non-protein carrier molecules, to which the second immunoglobulin partner may be operatively linked by conventional means.

10 Fusion or linkage between the second immunoglobulin partners, e.g., antibody sequences, and the effector agent may be by any suitable means, e.g., by conventional covalent or ionic bonds, protein fusions, or hetero-bifunctional cross-linkers, e.g., carbodiimide, glutaraldehyde, and the like. Such techniques are known in the art and readily described in conventional chemistry and biochemistry texts.

15 Additionally, conventional linker sequences which simply provide for a desired amount of space between the second immunoglobulin partner and the effector agent may also be constructed into the altered immunoglobulin coding region. The design of such linkers is well known to those of skill in the art.

In addition, signal sequences for the molecules of the invention may be modified to enhance expression.

20 An exemplary altered antibody contains a variable heavy and/or light chain peptide or protein sequence having the antigen specificity of mAb 2C10, e.g., the V_H and V_L chains. Still another desirable altered antibody of this invention is characterized by the amino acid sequence containing at least one, and preferably all of the CDRs of the variable region of the heavy and/or light chains of the rat antibody molecule 2C10 with the remaining sequences being derived from a human source, or a functional fragment or analog thereof.

25 In still a further embodiment, the engineered antibody of the invention may have attached to it an additional agent. For example, the procedure of recombinant DNA technology may be used to produce an engineered antibody of the invention in which the Fc fragment or CH2 CH3 domain of a complete antibody molecule has been replaced by an enzyme or other detectable molecule (i.e., a polypeptide effector or reporter molecule).

30 The second immunoglobulin partner may also be operatively linked to a non-immunoglobulin peptide, protein or fragment thereof heterologous to the CDR-containing sequence, for example, having the antigen specificity of rat 2C10. The resulting protein may exhibit both anti-IL-18 antigen specificity and characteristics of the non-immunoglobulin upon expression. That fusion partner characteristic may be, e.g., a functional characteristic such as another binding or receptor domain, or a

therapeutic characteristic if the fusion partner is itself a therapeutic protein, or additional antigenic characteristics.

Another desirable protein of this invention may comprise a complete antibody molecule, having full length heavy and light chains, or any discrete 5 fragment thereof, such as the Fab or F(ab'), fragments, a heavy chain dimer, or any minimal recombinant fragments thereof such as an F_v or a single-chain antibody (SCA) or any other molecule with the same specificity as the selected donor mAb, e.g., mAb 2C10. Such protein may be used in the form of an altered antibody, or may be used in its unfused form.

10 Whenever the second immunoglobulin partner is derived from an antibody different from the donor antibody, e.g., any isotype or class of immunoglobulin framework or constant regions, an engineered antibody results. Engineered antibodies can comprise immunoglobulin (Ig) constant regions and variable framework regions from one source, e.g., the acceptor antibody, and one or more 15 (preferably all) CDRs from the donor antibody, e.g., the anti-IL-18 antibody described herein. In addition, alterations, e.g., deletions, substitutions, or additions, of the acceptor mAb light and/or heavy variable domain framework region at the nucleic acid or amino acid levels, or the donor CDR regions may be made in order to retain donor antibody antigen binding specificity.

20 Such engineered antibodies are designed to employ one (or both) of the variable heavy and/or light chains of the IL-18 mAb (optionally modified as described) or one or more of the below-identified heavy or light chain CDRs. The engineered antibodies would be expected to be are neutralizing, i.e., they desirably block binding to the receptor of the IL-18 protein and they also block or prevent 25 proliferation of IL-18 dependent cells.

Such engineered antibodies may include a humanized antibody containing the framework regions of a selected human immunoglobulin or subtype, or a chimeric antibody containing the human heavy and light chain constant regions fused to the IL-18 antibody functional fragments. A suitable human (or other 30 animal) acceptor antibody may be one selected from a conventional database, e.g., the KABAT® database, Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant 35 region and/or a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable framework regions may be selected in a similar manner. It should be noted

that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody.

Desirably the heterologous framework and constant regions are selected from human immunoglobulin classes and isotypes, such as IgG (subtypes 1 through 4).

5 IgM, IgA, and IgE. However, the acceptor antibody need not comprise only human immunoglobulin protein sequences. For instance a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding a non-immunoglobulin amino acid sequence such as a polypeptide effector or reporter molecule.

10 One example of a particularly desirable humanized antibody would contain CDRs of 2C10 inserted onto the framework regions of a selected human antibody sequence. For neutralizing humanized antibodies, one, two or preferably three CDRs from the IL-18 antibody heavy chain and/or light chain variable regions are inserted into the framework regions of the selected human antibody sequence, 15 replacing the native CDRs of the latter antibody.

Preferably, in a humanized antibody, the variable domains in both human heavy and light chains have been engineered by one or more CDR replacements. It is possible to use all six CDRs, or various combinations of less than the six CDRs. Preferably all six CDRs are replaced. It is possible to replace the CDRs only in the 20 human heavy chain, using as light chain the unmodified light chain from the human acceptor antibody. Still alternatively, a compatible light chain may be selected from another human antibody by recourse to the conventional antibody databases. The remainder of the engineered antibody may be derived from any suitable acceptor human immunoglobulin.

25 The engineered humanized antibody thus preferably has the structure of a natural human antibody or a fragment thereof, and possesses the combination of properties required for effective therapeutic use, e.g., treatment of IL-18 mediated inflammatory diseases in man, or for diagnostic uses.

As another example, an engineered antibody may contain CDRs of the 30 variable light chain region of 2C10 and CDRs of the variable heavy chain region of 13G9. The resulting humanized antibody should be characterized by the same antigen binding specificity and high affinity of mAb 2C10.

It will be understood by those skilled in the art that an engineered antibody may be further modified by changes in variable domain amino acids without 35 necessarily affecting the specificity and high affinity of the donor antibody (i.e., an analog). It is anticipated that heavy and light chain amino acids may be substituted by other amino acids either in the variable domain frameworks or CDRs or both.

In addition, the constant region may be altered to enhance or decrease selective properties of the molecules of the instant invention. For example, dimerization, binding to Fc receptors, or the ability to bind and activate complement (see, e.g., Angal et al., *Mol. Immunol.* 30:105-108 (1993), Xu et al., *J. Biol. Chem.*, 5 *269*:3469-3474 (1994), Winter et al., EP 307,434-B).

An altered antibody which is a chimeric antibody differs from the humanized antibodies described above by providing the entire non-human donor antibody heavy chain and light chain variable regions, including framework regions, in association with human immunoglobulin constant regions for both chains. It is anticipated that 10 chimeric antibodies which retain additional non-human sequence relative to humanized antibodies of this invention may elicit a significant immune response in humans.

Such antibodies could be useful in the prevention and treatment of IL-18 mediated disorders, as discussed below.

15

VI. Production Of Altered Antibodies And Engineered Antibodies

Preferably, the variable light and/or heavy chain sequences and the CDRs of mAb 2C10 or other suitable donor mAbs, and their encoding nucleic acid sequences, are utilized in the construction of altered antibodies, preferably humanized 20 antibodies, of this invention, by the following process. The same or similar techniques may also be employed to generate other embodiments of this invention.

A hybridoma producing a selected donor mAb, e.g., the rat antibody 2C10, is conventionally cloned, and the DNA of its heavy and light chain variable regions obtained by techniques known to one of skill in the art, e.g., the techniques described 25 in Sambrook et al., (*Molecular Cloning (A Laboratory Manual)*, 2nd edition, Cold Spring Harbor Laboratory (1989)). The variable heavy and light regions of 2C10 containing at least the CDR-encoding regions and those portions of the acceptor mAb light and/or heavy variable domain framework regions required in order to retain donor mAb binding specificity, as well as the remaining immunoglobulin-derived parts of the antibody chain derived from a human immunoglobulin can be 30 obtained using polynucleotide primers and reverse transcriptase. The CDR-encoding regions are identified using a known database and by comparison to other antibodies.

A rat/human chimeric antibody may then be prepared and assayed for 35 binding ability. Such a chimeric antibody contains the entire non-human donor antibody V_H and V_L regions, in association with human Ig constant regions for both chains.

A humanized antibody may be derived from the chimeric antibody, or preferably, made synthetically by inserting the donor mAb CDR-encoding regions from the heavy and light chains appropriately within the selected heavy and light chain framework. Alternatively, a humanized antibody of the invention may be 5 prepared using standard mutagenesis techniques. Thus, the resulting humanized antibody contains human framework regions and donor mAb CDR-encoding regions. There may be subsequent manipulation of framework residues. The resulting humanized antibody can be expressed in recombinant host cells, e.g., COS, CHO or myeloma cells. Other humanized antibodies may be prepared using this 10 technique on other suitable IL-18-specific, neutralizing, high affinity, non-human antibodies.

A conventional expression vector or recombinant plasmid can be produced by placing these coding sequences for the altered antibody in operative association with conventional regulatory control sequences capable of controlling the replication 15 and expression in, and/or secretion from, a host cell. Regulatory sequences include promoter sequences, e.g., CMV promoter, and signal sequences, which can be derived from other known antibodies. Similarly, a second expression vector can be produced having a DNA sequence which encodes a complementary antibody light or heavy chain. Preferably this second expression vector is identical to the first except 20 insofar as the coding sequences and selectable markers are concerned, so to ensure as far as possible that each polypeptide chain is functionally expressed. Alternatively, the heavy and light chain coding sequences for the altered antibody may reside on a single vector.

A selected host cell is co-transfected by conventional techniques with both 25 the first and second vectors (or simply transfected by a single vector) to create the transfected host cell of the invention comprising both the recombinant or synthetic light and heavy chains. The transfected cell is then cultured by conventional techniques to produce the engineered antibody of the invention. The humanized antibody which includes the association of both the recombinant heavy chain and/or 30 light chain is screened from culture by appropriate assay, such as ELISA or RIA. Similar conventional techniques may be employed to construct other altered antibodies and molecules of this invention.

Suitable vectors for the cloning and subcloning steps employed in the methods and construction of the compositions of this invention may be selected by 35 one of skill in the art. For example, the conventional pUC series of cloning vectors, may be used. One vector used is pUC19, which is commercially available from supply houses, such as Amersham (Buckinghamshire, United Kingdom) or

Pharmacia (Uppsala, Sweden). Additionally, any vector which is capable of replicating readily, has an abundance of cloning sites and selectable genes (e.g., antibiotic resistance), and is easily manipulated may be used for cloning. Thus, the selection of the cloning vector is not a limiting factor in this invention.

5 Similarly, the vectors employed for expression of the engineered antibodies according to this invention may be selected by one of skill in the art from any conventional vector. The vectors also contain selected regulatory sequences (such as CMV promoters) which direct the replication and expression of heterologous DNA sequences in selected host cells. These vectors contain the above described DNA
10 sequences which code for the engineered antibody or altered immunoglobulin coding region. In addition, the vectors may incorporate the selected immunoglobulin sequences modified by the insertion of desirable restriction sites for ready manipulation.

15 The expression vectors may also be characterized by genes suitable for amplifying expression of the heterologous DNA sequences, e.g., the mammalian dihydrofolate reductase gene (DHFR). Other preferable vector sequences include a poly A signal sequence, such as from bovine growth hormone (BGH) and the betaglobin promoter sequence (betaglopro). The expression vectors useful herein may be synthesized by techniques well known to those skilled in this art.

20 The components of such vectors, e.g. replicons, selection genes, enhancers, promoters, signal sequences and the like, may be obtained from commercial or natural sources or synthesized by known procedures for use in directing the expression and/or secretion of the product of the recombinant DNA in a selected host. Other appropriate expression vectors of which numerous types are known in
25 the art for mammalian, bacterial, insect, yeast, and fungal expression may also be selected for this purpose.

30 The present invention also encompasses a cell line transfected with a recombinant plasmid containing the coding sequences of the engineered antibodies or altered immunoglobulin molecules thereof. Host cells useful for the cloning and other manipulations of these cloning vectors are also conventional. However, most desirably, cells from various strains of *E. coli* are used for replication of the cloning vectors and other steps in the construction of altered antibodies of this invention.

35 Suitable host cells or cell lines for the expression of the engineered antibody or altered antibody of the invention are preferably mammalian cells such as CHO, COS, a fibroblast cell (e.g., 3T3), and myeloid cells, and more preferably a CHO or a myeloid cell. Human cells may be used, thus enabling the molecule to be modified with human glycosylation patterns. Alternatively, other eukaryotic cell lines may be

employed. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Sambrook *et al.*, cited above.

Bacterial cells may prove useful as host cells suitable for the expression of the recombinant Fabs of the present invention (see, e.g., Plückthun, A., Immunol. Rev., 130:151-188 (1992)). However, due to the tendency of proteins expressed in bacterial cells to be in an unfolded or improperly folded form or in a non-glycosylated form, any recombinant Fab produced in a bacterial cell would have to be screened for retention of antigen binding ability. If the molecule expressed by the bacterial cell was produced in a properly folded form, that bacterial cell would be a desirable host. For example, various strains of *E. coli* used for expression are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Streptomyces*, other bacilli and the like may also be employed in this method.

Where desired, strains of yeast cells known to those skilled in the art are also available as host cells, as well as insect cells, e.g. *Drosophila* and *Lepidoptera* and viral expression systems. See, e.g. Miller *et al.*, Genetic Engineering, 8:277-298, Plenum Press (1986) and references cited therein.

The general methods by which the vectors of the invention may be constructed, the transfection methods required to produce the host cells of the invention, and culture methods necessary to produce the altered antibody of the invention from such host cell are all conventional techniques. Likewise, once produced, the altered antibodies of the invention may be purified from the cell culture contents according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Such techniques are within the skill of the art and do not limit this invention.

Yet another method of expression of the humanized antibodies may utilize expression in a transgenic animal, such as described in U.S. Patent No. 4,873,316. This relates to an expression system using the animal's casein promoter which when transgenically incorporated into a mammal permits the female to produce the desired recombinant protein in its milk.

Once expressed by the desired method, the engineered antibody is then examined for *in vitro* activity by use of an appropriate assay. Presently conventional ELISA assay formats are employed to assess qualitative and quantitative binding of the engineered antibody to IL-18. Additionally, other *in vitro* assays may also be used to verify neutralizing efficacy prior to subsequent human clinical studies

performed to evaluate the persistence of the engineered antibody in the body despite the usual clearance mechanisms.

Following the general procedures described for preparing humanized antibodies, one of skill in the art may also construct humanized antibodies from 5 other donor IL-18 antibodies, variable region sequences and CDR peptides described herein. Engineered antibodies can be produced with variable region frameworks potentially recognized as "self" by recipients of the engineered antibody. Minor modifications to the variable region frameworks can be implemented to effect large increases in antigen binding without appreciable increased immunogenicity for the 10 recipient. Such engineered antibodies may effectively treat a human for IL-18 mediated conditions. Such antibodies may also be useful in the diagnosis of such conditions.

VII. Therapeutic/Prophylactic Uses

15 This invention also relates to a method of treating humans experiencing autoimmune related symptoms, such as MS, which comprises administering an effective dose of antibodies including one or more of the engineered antibodies or altered antibodies described herein, or fragments thereof.

20 The therapeutic response induced by the use of the molecules of this invention is produced by the binding to human IL-18 and thus subsequently blocking Th1 stimulation. Thus, the molecules of the present invention, when in preparations and formulations appropriate for therapeutic use, are highly desirable for those persons experiencing autoimmune disease, such as but not limited to MS, RA, IDDM, IBD and psoriasis.

25 The altered antibodies, antibodies and fragments thereof of this invention may also be used in conjunction with other antibodies, particularly human mAbs reactive with other markers (epitopes) responsible for the condition against which the engineered antibody of the invention is directed.

30 The therapeutic agents of this invention are believed to be desirable for treatment of autoimmune conditions from about 2 days to 6 months or as needed. For example, longer treatments may be desirable when treating MS or the like. The dose and duration of treatment relates to the relative duration of the molecules of the present invention in the human circulation, and can be adjusted by one of skill in the art depending upon the condition being treated and the general health of the patient.

35 The mode of administration of the therapeutic agent of the invention may be any suitable route which delivers the agent to the host. The altered antibodies, antibodies, engineered antibodies, and fragments thereof, and pharmaceutical

compositions of the invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly, intravenously, or intranasally.

Therapeutic agents of the invention may be prepared as pharmaceutical compositions containing an effective amount of the engineered (e.g., humanized) antibody of the invention as an active ingredient in a pharmaceutically acceptable carrier. In the prophylactic agent of the invention, an aqueous suspension or solution containing the engineered antibody, preferably buffered at physiological pH, in a form ready for injection is preferred. The compositions for parenteral administration will commonly comprise a solution of the engineered antibody of the invention or a cocktail thereof dissolved in an pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, e.g., 0.4% saline, 0.3% glycine, and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques (e.g., filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antibody of the invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and between about 1 ng to about 100 mg, e.g. about 50 ng to about 30 mg or more preferably, about 5 mg to about 25 mg, of an engineered antibody of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 to about 30 and preferably 5 mg to about 25 mg of an engineered antibody of the invention. Actual methods for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania.

It is preferred that the therapeutic agent of the invention, when in a pharmaceutical preparation, be present in unit dose forms. The appropriate therapeutically effective dose can be determined readily by those of skill in the art. 35 To effectively treat an inflammatory disorder in a human or other animal, one dose of approximately 0.1 mg to approximately 20 mg per 70 kg body weight of a protein or an antibody of this invention should be administered parenterally, preferably *i.v.*

or i.m. (intramuscularly). Such dose may, if necessary, be repeated at appropriate time intervals selected as appropriate by a physician during the disease.

The altered antibodies and engineered antibodies of this invention may also be used in diagnostic regimens, such as for the determination of IL-18 mediated disorders or tracking progress of treatment of such disorders. As diagnostic reagents, these altered antibodies may be conventionally labeled for use in ELISA's and other conventional assay formats for the measurement of IL-18 levels in serum, plasma or other appropriate tissue, or the release by human cells in culture. The nature of the assay in which the altered antibodies are used are conventional and do not limit this disclosure.

Thus, one embodiment of the present invention relates to a method for aiding the diagnosis of autoimmune disease and other conditions associated with excess Th1 T cell production in a patient which comprises the steps of determining the amount of human IL-18 in sample (plasma or tissue) obtained from said patient and comparing said determined amount to the mean amount of human IL-18 in the normal population, whereby the presence of a significantly elevated amount of IL-18 in the patient's sample is an indication autoimmune disease and other conditions associated with excess Th1 T cell production.

The antibodies, altered antibodies or fragments thereof described herein can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and art-known lyophilization and reconstitution techniques can be employed.

The following examples illustrate various aspects of this invention including the construction of exemplary engineered antibodies and expression thereof in suitable vectors and host cells, and are not to be construed as limiting the scope of this invention. All amino acids are identified by conventional three letter or single letter codes. All necessary restriction enzymes, plasmids, and other reagents and materials were obtained from commercial sources unless otherwise indicated. All general cloning legation and other recombinant DNA methodology were as performed in T. Maniatis *et al.*, cited above, or the second edition thereof (1989), eds. Sambrook *et al.*, by the same publisher ("Sambrook *et al.*").

Example 1 - Production of MAbs to IL-18

A. Monoclonal antibody generation

Mice (F1 hybrids of Balb/c and C57BL/6) or rats (Sprague Dawley) were immunised with 30 µg recombinant IL-18 in adjuvant and 4 weeks later with 30 µg

IL-18 in adjuvant. On the basis of a good serum antibody titre to IL-18 animals received a further immunization of 10-30 µg IL-18 (i.p. in saline). Three days following the final immunization a splenectomy was performed. Mouse or rat spleen cells were used to prepare hybridomas by standard procedures. (Zola, H.Ed., 5 Monoclonal Antibodies. CRC Press Inc. 1987). Positive hybridomas were cloned by the limiting dilution method.

B. Purification of Mabs

Mabs were purified by ProsepA (Bio Processing, Consett, UK) chromatography respectively using the manufacturer's instructions. Mabs were 10 >95% pure by SDS-PAGE.

C. Isotyping of Mabs

All rat and mouse Mabs were isotype by commercially available kits (Zymed, Amersham) and found to be IgG1 kappa.

15

Example 2 – Assays

A. Biotinylation Of IL-18

IL-18 was biotinylated using a kit purchased from Molecular Probes Inc. using a 10:1 ratio of biotinylation reagent. Biotinylation had no effect on the 20 biological activity of IL-18

B. Hybridoma Screening Assay

96-well plates were coated with streptavidin (2µg/ml, 100µl/well in PBS) by incubation overnight at 4°C. The solution was then aspirated and non-specific 25 binding sites were blocked with 250µl/well of 1% bovine serum albumin (BSA) in TBS buffer (50mM Tris, 150 mM NaCl, 0.02% Kathon, pH 7.4) for 5-60 minutes at RT. Following this and each of the following steps, the plate was washed 4 times in wash buffer (10 mM Tris, 150 mM NaCl, 0.05% Tween 20, 0.02% Kathon, pH 7.4). To each well, 100 µL biotin IL-18 (100ng/ml) in assay buffer (0.5% BSA, 0.05% 30 bovine gamma globulin, 0.01% Tween 40, 20µM diethylenetriaminepentaacetic in TBS buffer) was added and the plates were incubated for 30 min at RT in a shaker-incubator. To each well 50µl hybridoma medium and 50 µl assay buffer was then

added and incubated for 60 min at RT in a shaker-incubator. To each well was then added 100ul 0.5 μ g/ml Eu³⁺ labelled anti-mouse or anti-rat antibody in assay buffer. Finally 200 μ l /well of enhancer (Wallac) was added and incubated for 5 min at RT and the time-resolved fluorescence measured. Hybridomas having counts >100K 5 were expanded into 24-well plates.

C. Immunoassay

To determine the specificity of the anti-IL-18 Mabs) generated 96-well plates were coated, blocked and incubated with biotin IL-18 as above. All the following 10 incubations were performed in a shaker-incubator at RT. After washing the wells 50 μ l IL-18 (3 μ g/ml) or assay buffer and 50 μ l Mab were added and incubated for 60 min. After washing the wells 100ul 0.5 μ g/ml Eu³⁺ labelled anti-mouse or anti-rat antibody in assay buffer was added for 60 min, the wells washed and then 100 μ l /well of enhancer (Wallac) was added and incubated for 5 min at RT and the time-15 resolved fluorescence measured. All positive hybridomas showed displacement of binding with IL-18.

D. Neutralization Assay

PBMC from healthy donors were isolated by Ficol-Paque (Pharmacia) 20 gradient and cultured in 96 well plates in 10% FBS DMEM/F12 media with 1 μ g/ml ConA (Sigma) in the presence of IL-18 (5ng/ml) and/or Mabs. After 18h culture at 37°C, 5% CO₂ in air, 90% humidity 25 μ l media was removed and interferon gamma (IFNg) concentration measured by immunoassay. The results, obtained from an average of three experiments, are summarized in Table I.

25

E. Affinity Measurements Of Monoclonal Antibody

The affinity of the purified mAbs was measured in the BIACore optical biosensor (Pharmacia Biosensor, Uppsala, Sweden) using a flow rate of 30ul/min. Kinetic data was evaluated using relationships described previously (Karlsson et al, 30 J. Immunol. Meth., 145:229-240 (1991) and which is incorporated by reference in its entirely. The mAb (diluted in HBS buffer, 10 mM HEPES, 150 mM NaCl, 0.01%

Tween-20, pH 7.4) was injected over a rabbit anti-mouse IgG Fc or goat anti-rat IgG Fc surface, followed by buffer flow and the RU was recorded. IL-18 (diluted in HBS buffer) was then injected for 180 seconds followed by a buffer flow for 500 seconds and the RU was recorded. The sensor chip surface was regenerated by an injection of 5 0.1 M phosphoric acid. The on-rates (Kass) and off-rates (Kdiss) of binding were calculated using BIACore software and together these yield a calculated equilibrium constant (K_D) of 12×10^{-9} M for mAb 13G9, 3.9×10^{-11} M for mAb 2C10 and 1.5×10^{-10} M for mAb 14B7. See Table I.

10 F. *Epitope Analysis Of Monoclonal Antibody*

The epitope analysis of the purified Mabs was measured in the BIACore. Using a flow rate of 10 μ l/min, the first Mab (diluted in HBS buffer) was injected over a rabbit anti-mouse IgG Fc or goat anti-rat IgG Fc surface, followed by an injection of IL-18 for 240s, an injection of blocking Mabs for 48s and an injection of 15 the second Mab for 240s. The surface was regenerated by an injection of 0.1M phosphoric acid and the RU was recorded after each injection. It was found that Mabs 13G9, 2C10 and 14B7 have similar or overlapping epitopes.

TABLE I

20 Affinity and neutralizing activity of mAbs reactive with human IL-18

mAb	Kd (pM) ^a	Neutralisation IC50 (nM) ^b
2C10 (rat)	39	0.1
25 14B7 (rat)	150	0.2
13G9 (mouse)	12000	3.0

^a Determined by optical biosensor (BIACore) analysis (25°C)

^b Inhibition of IFN gamma production (in nM) of PBMC in response to 5ng/ml human IL-18

Example 3 – CDR sequences*Gene Cloning and Sequence Analysis:*

The variable heavy and light genes were cloned from hybridoma cells using
5 standard molecular biological methods described briefly as follows. Total RNA was
isolated from the hybridoma cells using TRIzol Reagent (Life Technologies Cat. #
15596-026) according to manufacturer's protocol. The RNA was reverse transcribed
with a RT-PCR kit per the manufacturer's instructions (Boehringer Mannheim Cat.
No. 1483-188) using a poly-dT oligonucleotide for priming. Following first strand
10 cDNA synthesis, the heavy and light V regions were PCR amplified using 3'
constant region specific primers and degenerate 5' primers. The degenerate 5' primer
sequences were designed to encode the previously determined N terminal amino acid
sequences of the variable heavy or light chain regions. Full length sequences from
multiple clones were obtained from each PCR amplification and aligned to provide
15 consensus. Accordingly, the first 17 bases of DNA sequence for both the heavy and
light chains are PCR primer generated, however the translated protein sequence is
native.

The nucleotide and deduced amino acid sequences for hybridoma antibodies
2C10, 13G9, and 14B7 are shown Figures 1-6. In each case the CDRs and the
20 nucleotide sequences encoding them are *boxed*. The degenerate primer sequences
are *bolded*.

WHAT IS CLAIMED IS:

1. A rodent neutralizing monoclonal antibody specific for human interleukin-18 and having a binding affinity characterized by a dissociation constant equal to or less than about 3.9×10^{-11} M.
2. The monoclonal antibody according to claim 1 which is a rat monoclonal antibody.
- 10 3. The monoclonal antibody according to claim 1 which is a murine monoclonal antibody.
4. The monoclonal antibody according to claim 2 which comprises the light chain amino acid sequence of SEQ ID NO: 1, and the heavy chain amino acid sequence of SEQ ID NO: 9.
- 15 5. The monoclonal antibody according to claim 3 which comprises the light chain amino acid sequence of SEQ ID NO: 17 and the heavy chain amino acid sequence of SEQ ID NO: 25.
- 20 6. The monoclonal antibody according to claim 2 which comprises the light chain amino acid sequence of SEQ ID NO: 33 and the heavy chain amino acid sequence of SEQ ID NO: 41 .
- 25 7. The monoclonal antibody according to claim 1 having the identifying characteristics of 2C10, 14B7 or 13G9.
- 30 8. A hybridoma which produces the monoclonal antibody of claim 4.
9. A hybridoma which produces the monoclonal antibody of claim 5.
10. A hybridoma which produces the monoclonal antibody of claim 6.
- 35 11. A hybridoma having the identifying characteristics of cell line 19522C10(2)F2(1)A1, 195214B7(1)H10 and 187413G9(3)F12

12. A neutralizing Fab fragment or F(ab')₂ fragment thereof, produced by deleting the Fc region of the monoclonal antibody of claim 1.

5 13. An altered antibody comprising a heavy chain and a light chain, wherein the framework regions of said heavy and light chains are derived from at least one selected antibody and the amino acid sequences of the complementarity determining regions of each said chain are derived from the monoclonal antibody of claim 1.

10 14. An immunoglobulin light chain complementarity determining region (CDR), the amino acid sequence of which is selected from the group consisting of:

- (a) SEQ ID NO: 3
- (b) SEQ ID NO: 5
- 15 (c) SEQ ID NO: 7
- (d) SEQ ID NO: 19
- (e) SEQ ID NO: 21
- (f) SEQ ID NO: 23
- (g) SEQ ID NO: 35
- 20 (h) SEQ ID NO: 37
- (i) SEQ ID NO: 39

15. An immunoglobulin heavy chain complementarity determining region (CDR), the amino acid sequence of which is selected from the group consisting of:

- (a) SEQ ID NO: 11
- (b) SEQ ID NO: 13
- (c) SEQ ID NO: 15
- (d) SEQ ID NO: 27
- 30 (e) SEQ ID NO: 29
- (f) SEQ ID NO: 31
- (g) SEQ ID NO: 43
- (h) SEQ ID NO: 45
- (i) SEQ ID NO: 47

35 16. A nucleic acid molecule encoding the immunoglobulin complementarity determining region (CDR) of claim 14.

17. A nucleic acid molecule encoding the immunoglobulin complementarity determining region (CDR) of claim 15.

5 18. A pharmaceutical composition comprising the altered antibody of claim 13 and a pharmaceutically acceptable carrier.

10 19. A method of treating conditions associated with autoimmune disease comprising the step of administering to said human in need thereof an effective amount of the altered antibody of claim 13.

20. The method of claim 19 where said disease is multiple sclerosis.

15 21. The method of claim 19 where said disease is rheumatoid arthritis type 1 or insulin dependent diabetes.

20 22. The method of claim 19 where said disease is inflammatory bowel disease.

23. The method of claim 19 where said disease is psoriasis.

24. An isolated nucleic acid sequence which is selected from the group consisting of:

25 (a) a nucleic acid sequence encoding the altered antibody of claim 13
(b) a nucleic acid sequence complementary to (a); and
(c) a fragment or analog of (a) or (b), which encodes a protein,
characterized by having a specificity for human interleukin-18;
wherein said sequence optionally contains a restriction site.

30 25. A method to assess the presence or absence of human IL-18 in a human which comprises obtaining a sample of biological fluid from a patient and allowing the monoclonal antibody of claim 1 to come in contact with such sample under conditions such that an IL-18/monoclonal antibody complex can form and detecting the presence or absence of said IL-18/monoclonal antibody complex.

26. A method for aiding in the diagnosis of autoimmune disease associated with comprising the steps of determining the amount of human IL-18 in a sample of a patient according to the method of claim 25 and comparing that to the mean amount of human IL-18 in the normal population, whereby the presence of
5 significantly elevated amount of human IL-18 in the patient is an indication of autoimmune disease.

V_k2c10

1 0 30
 1 D I Q M T Q S P A S L S A S L 15
GAC ATT CAA ATG ACC CAG TCT CCA GCT TCC CTG TCT GCA TCT CTG
 CTG TAA GTT TAC TGG GTC AGA GGT CGA AGG GAC AGA CGT AGA GAC

CDRI

50 7 0 90
 16 G E T V S I E C L A S E D I Y 30
GGA GAA ACT GTC TCC ATC GAA TGT CTG GCA AGT GAG GAC ATA TAC
 CCT CTT TGA CAG AGG TAG CTT ACA GAC CGT TCA CTC CTG TAT ATG

1 10 13 0
 31 T Y L T W Y Q Q K P G K S P Q 45
ACT TAT TTA ACA TGG TAT CAG CAG AAA CCA GGG AAA TCT CCT CAA
 TGA ATA AAT TGT ACC ATA GTC GTC TTT GGT CCC TTT AGA GGA GTT

CDRII

150 1 70
 46 L L I Y G A N K L Q D G V P S 60
CTC CTG ATC TAT GGT GCA AAT AAG TTG CAA GAT GGG GTC CCA TCA
 GAG GAC TAG ATA CCA CGT TTA TTC AAC GTT CTA CCC CAG GGT AGT

19 0 210
 61 R F S G S G S G T Q Y S L K I 75
CGG TTC AGT GGC AGT GGA TCT GGC ACA CAG TAT TCT CTC AAG ATC
 GCC AAG TCA CCG TCA CCT AGA CCG TGT GTC ATA AGA GAG TTC TAG

2 30 25 0 270
 76 S G I Q P E D E G D Y F C L Q 90
AGC GGC ATA CAA CCT GAA GAT GAA GGG GAT TAT TTC TGT CTA CAG
 TCG CCG TAT GTT GGA CTT CTA CTT CCC CTA ATA AAG ACA GAT GTC

CDRIII

2 90 31 0
 91 G S K F P L T F G S G T K L E 105
GGT TCC AAG TTT CCG CTC ACG TTC GGT TCT GGG ACC AAG CTG GAG
 CCA AGG TTC AAA GGC GAG TGC AAG CCA AGA CCC TGG TTC GAC CTC

106 I K R 108
 ATC AAA CGG
 TAG TTT GCC

FIGURE 1

1/6

VH2C10

1 10 30
 1 E V Q L Q Q S G A E L V R P G 15
GAG GTC CAG CTA CAG CAG TCT GGG GCT GAG CTT GTG AGA CCT GGG
 CTC CAG GTC GAT GTC AGA CCC CGA CTC GAA CAC TCT GGA CCC

16 50 70 90
 16 T S V K L S C K V S G E I S T 30
 ACC TCT GTG AAG TTA TCT TGC AAA GTT TCT GGC GAA ATA AGT ACA
 TGG AGA CAC TTC AAT AGA ACG TTT CAA AGA CCG CTT TAT TCA TGT

CDRI

31 1 10 13 0
 31 G Y Y F H F V R R R P G Q G L 45
GGA TAC TAT TTC CAC TTT GTG AGG CGA AGG CCT GGA CAG GGT CTG
CCT ATG ATA AAG GTG AAA CAC TCC GCT TCC GGA CCT GTC CCA GAC

CDRII

46 150 1 70
 46 E W I G R I D P E D D S T K Y 60
 GAA TGG ATA GGA AGG ATT GAT CCT GAG GAT GAT AGT ACT AAA TAT
 CTT ACC TAT CCT TCC TAA CTA GGA CTC CTA CTA TCA TGA TTT ATA

61 19 0 210
 61 A E R F K D R A T L T A Q T S 75
GCT GAG AGG TTC AAA GAC AGG GCG ACG CTC ACT GCA CAA ACA TCC
CGA CTC TCC AAG TTT CTG TCC CGC TGC GAG TGA CGT GTT TGT AGG

76 2 30 25 0 270
 76 S N T A Y L N L S S L T S E D 90
 TCC AAC ACA GCC TAC CTG AAC CTC AGC AGC CTG ACC TCT GAG GAC
 AGG TTG TGT CGG ATG GAC TTG GAG TCG TCG GAC TGG AGA CTC CTG

91 2 90 31 0
 91 T A T Y F C T T W R I Y R D S 105
 ACT GCA ACT TAT TTT TGT ACC ACA TGG CGG ATA TAC CGA GAT AGT
 TGA CGT TGA ATA AAA ACA TGG TGT ACC GCC TAT ATG GCT CTA TCA

CDRIII

106 330 3 50
 106 S G R P F Y V M D A W G Q G A 120
 TCT GGC CGC CCC TTC TAT GTT ATG GAT GCC TGG GGT CAA GGA GCT
 AGA CCG GCG GGG AAG ATA CAA TAC CTA CGG ACC CCA GTT CCT CGA

121 37 0 126
 121 S V T V S S
 TCA GTC ACT GTC TCC TCA
 AGT CAG TGA CAG AGG AGT

FIGURE 2
2/6

V_k13g9

1 D V V M T Q T P L S L P V S L 15
 1 GAC GTT ATG ACT CAA ACT CCT CTC TCC CTG CCT GTC AGT CTT
 CTG CAA CAA TAC TGA GTT TGA GGA GAG AGG GAC GGA CAG TCA GAA

30

16 G D Q A S I S C R S S Q S L V 90
 GGA GAT CAA GCC TCC ATC TCT TGC AGA TCT AGT CAG AGC CTT GTC
 CCT CTA GTT CGG AGG TAG AGA ACG TCT AGA TCA GTC TCG GAA CAT 30

50 70

31 H S N G N T Y L H W Y L Q K P 45
 CAC AGT AAT GGA AAC ACC TAT TTA CAT TGG TAC CTG CAG AAG CCA
 GTG TCA TTA CCT TTG TGG ATA AAT GTA ACC ATG GAC GTC TTC GGT

1 10 13 0

46 G Q S P K L L I Y K V S N R F 60
 GGC CAG TCT CCA AAG CTC CTG ATC TAC AAA GTT TCC AAC CGA TTT
 CCG GTC AGA GGT TTC GAG GAC TAG ATG TTT CAA AGG TTG GCT AAA

150 1 70

61 S G V P D R F S G S G S G T D 75
 TCT GGG GTC CCA GAC AGG TTC AGT GGC AGT GGA TCA GGT ACA GAT
 AGA CCC CAG GGT CTG TCC AAG TCA CCG TCA CCT AGT CCA TGT CTA

19 0 210

76 F T L K I S R V E A E D L G V 90
 TTC ACA CTC AAG ATC AGC AGA GTG GAG GCT GAG GAT CTG GGA GTT
 AAG TGT GAG TTC TAG TCG TCT CAC CTC CGA CTC CTA GAC CCT CAA

2 30 25 0 270

91 Y F C S Q S T H V P P Y T F G 105
 TAT TTC TGC TCT CAA AGT ACA CAT GTT CCT CCG TAC ACG TTC GGA
 ATA AAG ACG AGA GTT TCA TGT GTA CAA GGA GGC ATG TGC AAG CCT

31 0

106 G G T K L E I K R 114
 GGG GGG ACC AAG CTG GAA ATA AAA CGG
 CCC CCC TGG TTC GAC CTT TAT TTT GCC

330

FIGURE 3
3/6

VH13G9

1 0 30 15

1 Q V T L K E S G P G I L K P S
CAA GTT ACT CTT AAG GAG TCT GGC CCT GGG ATA TTG AAG CCC TCA
 GTT CAA TGA GAA TTC CTC AGA CCG GGA CCC TAT AAC TTC GGG AGT

50 70 90 30

16 Q T L S L T C S F S G F S L S
 CAG ACC CTC AGT CTG ACT TGT TCT TTC TCT GGG TTT TCT CTG AGC
 GTC TGG GAG TCA GAC TGA ACA AGA AAG AGA CCC AAA AGA GAC TCG

1 10 13 0 45

31 T S G M G I A W V R Q P S G K
ACT TCT GGT ATG GGT ATT GCC TGG GTT CGT CAG CCT TCA GGG AAG
 TGA AGA CCA TAC CCA TAA CGG ACC CAA GCA GTC GGA AGT CCC TTC

150 1 70 60

46 G L E W L A D I W W D D N K Y
 GGT CTG GAG TGG CTG GCA GAC ATT TGG TGG GAT GAT AAT AAG TAT
 CCA GAC CTC ACC GAC CGT CTG TAA ACC ACC CTA CTA TTA TTC ATA

19 0 210 75

61 Y N P S L E S Q L T I S K D T
 TAT AAT CCA TCC CTG GAG AGC CAG CTC ACA ATC TCC AAG GAT ACC
 ATA TTA GGT AGG GAC CTC TCG GTC GAG TGT TAG AGG TTC CTA TGG

2 30 25 0 270 90

76 S R N Q V F L T I T S V D T A
 TCC AGA AAC CAG GTA TTC CTC ACG ATC ACC AGT GTG GAC ACT GCA
 AGG TCT TTG GTC CAT AAG GAG TGC TAG TGG TCA CAC CTG TGA CGT

2 90 31 0 105

91 D S A T Y Y C A R H H Y D G S
 GAT TCT GCC ACT TAT TAC TGT GCT CGT CAT CAT TAC GAC GGT AGT
 CTA AGA CGG TGA ATA ATG ACA CGA GCA GTA GTA ATG CTG CCA TCA

330 3 50 120

106 S L L P M D Y W G Q G T S V T
 AGC CTC CTG CCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC ACC
 TCG GAG GAC GGA TAC CTG ATG ACC CCA GTT CCT TGG AGT CAG TGG

121 V S S 123
 GTC TCC TCA
 CAG AGG AGT

FIGURE 4
4/6

Vkl4B7

1	1 0	30
1	D I Q M T Q S P A S L S A S L	
	GAT ATT CAA ATG ACG CAG TCT CCA GCT TCC CTG TCT GCA TCT CTG	
	CTA TAA GTT TAC TGC GTC AGA GGT CGA AGG GAC AGA CGT AGA GAC	
16 50		
16	E T V S I E C	7 0 CDR I 90
	GGA GAA ACT GTC TCC ATC GAA TGT	CTA GCA AGT GAG GAC ATA TAC
	CCT CTT TGA CAG AGG TAG CTT ACA	GAT CGT TCA CTC CTG TAT ATG
31 1 10 13 0		
31	S Y L A W Y Q Q K P G K S P Q	
	AGT TAT TTA GCA TGG TAT CAA CAG AAG CCA GGG AAA TCT CCT CAG	
	TCA ATA AAT CGT ACC ATA GTT GTC TTC GGT CCC TTT AGA GGA GTC	
46 150 CDR II 1 70		
46	L L I Y A T K R L Q D	
	CTC CTG ATC TAT GCC ACA AAA AGG TTG CAA GAT	G G V P S
	GAG GAC TAG ATA CGG TGT TTT TCC AAC GTT CTA	CCC CAG GGT AGT
61 19 0 210		
61	R F S G S G S G T Q Y S L K I	
	CGG TTC AGT GGC AGT GGA TCT GGC ACA CAG TAT TCT CTC AAA ATA	
	GCC AAG TCA CCG TCA CCT AGA CCG TGT GTC ATA AGA GAG TTT TAT	
76 2 30 25 0 270		
76	S D M Q P E D E G D Y F C	
	AGC GAC ATG CAA CCT GAA GAT GAA GGG GAT TAT TTC TGT	L Q CTA CAG
	TCG CTG TAC GTT GGA CTT CTA CTT CCC CTA ATA AAG ACA GAT GTC	GAT GTC
91 2 90 31 0		
91	N S K F P V T F G S G T K L E	
	AAT TCC AAG TTT CCG GTC ACG TTC GGT TCT GGG ACC AAG CTG GAG	
	TTA AGG TTC AAA GGC CAG TGC AAG CCA AGA CCC TGG TTC GAC CTC	
106 108		
106	I K R	ATC AAA CGG
	TAG TTT GCC	

FIGURE 5
5/6

VH14B7

1	E	V	Q	L	Q	Q	S	G	A	E	L	V	R	P	G	15
	GAG	GTT	CAG	CTT	CAG	CAG	TCT	GGG	GCT	GAG	CTT	GTG	AGA	CCT	GGG	
	CTC	CAA	GTC	GAA	GTC	GTC	AGA	CCC	CGA	CTC	GAA	CAC	TCT	GGA	CCC	
16	T	S	V	K	F	S	C	K	V	S	G	D	T	P	T	30
	ACC	TCT	GTG	AAG	TTT	TCT	TGC	AAA	GTT	TCT	GGC	GAT	ACC	CCT	ACA	
	TGG	AGA	CAC	TTC	AAA	AGA	ACG	TTT	CAA	AGA	CCG	CTA	TGG	GGA	TGT	
31	CDR I								1	10				13	0	45
	T	Y	Y	V	H	F	V	R	Q	R	P	G	Q	G	L	
	ACA	TAC	TAC	GTG	CAC	TTT	GTG	AGA	CAA	AGG	CCT	GGA	CAG	GGT	CTG	
	TGT	ATG	ATG	CAC	GTG	AAA	CAC	TCT	GTT	TCC	GGA	CCT	GTC	CCA	GAC	
46	E	W	I	G	R	I	D	P	E	D	T	S	T	K	Y	150
	GAA	TGG	ATA	GGA	AGG	ATT	GAT	CCT	GAG	GAT	ACT	AGT	ACT	AAA	TAT	
	CTT	ACC	TAT	CCT	TCC	TAA	CTA	GGA	CTC	CTA	TGA	TCA	TGA	TTT	ATA	
61	A	E	K	F	R	N	K	A	T	F	T	A	D	P	S	19 0
	GCT	GAG	AAG	TTC	AGA	AAT	AAG	GCG	ACA	TTC	ACT	GCA	GAT	CCA	TCC	
	CGA	CTC	TTC	AAG	TCT	TTA	TTC	CGC	TGT	AAG	TGA	CGT	CTA	GGT	AGG	
76	S	N	T	A	Y	L	N	L	S	S	L	T	P	E	D	210
	TCC	AAC	ACA	GCC	TAC	CTA	AAC	CTC	AGC	AGC	CTG	ACC	CCT	GAG	GAC	
	AGG	TTG	TGT	CGG	ATG	GAT	TTG	GAG	TCG	TCG	GAC	TGG	GGA	CTC	CTG	
91	T	A	T	Y	F	C	T	I	M	R	Y	H	S	T	Y	25 0
	ACT	GCA	ACC	TAT	TTT	TGT	ACC	ATA	ATG	CGG	TAC	CAT	AGT	ACC	TAT	
	TGA	CGT	TGG	ATA	AAA	ACA	TGG	TAT	TAC	GCC	ATG	GTA	TCA	TGG	ATA	
106	CDR III								2	90				31	0	105
	R	V	Y	V	M	D	F	W	G	Q	G	T	A	V	T	330
	AGG	GTC	TAT	GTG	ATG	GAT	TTC	TGG	GGT	CAA	GGA	ACT	GCA	GTC	ACT	
	TCC	CAG	ATA	CAA	TAC	CTA	AAG	ACC	CCA	GTT	CCT	TGA	CGT	CAG	TGA	
121	V	S													120	
	GTC	TCC	TC													
	CAG	AGG	AG													

FIGURE 6
6/6

SEQUENCE LISTING

<110> Holmes, Stephen D.
Ho, Yen Sen
Taylor, Alexander
Abdel-Meguid, Sherin S.

<120> Recombinant IL-18 Antagonists Useful in
Treatment of IL-18 Mediated Disorders

<130> P50897

<140> 60/125,299
<141> 1999-03-19

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gaa act gtc tcc atc gaa tgt ctg gca agt gag gac ata tac act tat 96
Glu Thr Val Ser Ile Glu Cys Leu Ala Ser Glu Asp Ile Tyr Thr Tyr
20 25 30

tta aca tgg tat cag cag aaa cca ggg aaa tct cct caa ctc ctg atc 144
 Leu Thr Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro Gln Leu Leu Ile
 35 40 45

tat ggt gca aat aag ttg caa gat ggg gtc cca tca cgg ttc agt ggc 192
 Tyr Gly Ala Asn Lys Leu Gln Asp Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

agt gga tct ggc aca cag tat tct ctc aag atc agc ggc ata caa cct 240
 Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Ser Gly Ile Gln Pro
 65 70 75 80

gaa gat gaa ggg gat tat ttc tgt cta cag ggt tcc aag ttt ccg ctc 288
 Glu Asp Glu Gly Asp Tyr Phe Cys Leu Gln Gly Ser Lys Phe Pro Leu
 85 90 95

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 Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg
 100 105

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 1 5 10 15
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 20 25 30
 Leu Thr Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro Gln Leu Leu Ile
 35 40 45
 Tyr Gly Ala Asn Lys Leu Gln Asp Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Ser Gly Ile Gln Pro
 65 70 75 80
 Glu Asp Glu Gly Asp Tyr Phe Cys Leu Gln Gly Ser Lys Phe Pro Leu
 85 90 95

Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg
 100 105

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 1 5 10

33

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 1 5 10

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 Gly Ala Asn Lys Leu Gln Asp
 1 5

21

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<400> 6
Gly Ala Asn Lys Leu Gln Asp
1 5

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<223> *VK2C10 Light Chain CDR III*

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Leu Gln Gly Ser Lys Phe Pro Leu Thr
1 5

27

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Leu Gln Gly Ser Lys Phe Pro Leu Thr
1 5

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<223> Heavy chain V region

<400> 9

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Glu	Val	Gln	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Arg	Pro	Gly	Thr	
1			5						10						15	

tct	gtg	aag	tta	tct	tgc	aaa	gtt	tct	ggc	gaa	ata	agt	aca	gga	tac	96
Ser	Val	Lys	Leu	Ser	Cys	Lys	Val	Ser	Gly	Glu	Ile	Ser	Thr	Gly	Tyr	
20				25										30		

tat	ttc	cac	ttt	gtg	agg	cga	agg	cct	gga	cag	ggt	ctg	gaa	tgg	ata	144
Tyr	Phe	His	Phe	Val	Arg	Arg	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile	
35				40										45		

gga	agg	att	gat	cct	gag	gat	gat	agt	act	aaa	tat	gct	gag	agg	ttc	192
Gly	Arg	Ile	Asp	Pro	Glu	Asp	Asp	Ser	Thr	Lys	Tyr	Ala	Glu	Arg	Phe	
50				55										60		

aaa	gac	agg	gcg	acg	ctc	act	gca	caa	aca	tcc	tcc	aac	aca	gcc	tac	240
Lys	Asp	Arg	Ala	Thr	Leu	Thr	Ala	Gln	Thr	Ser	Ser	Asn	Thr	Ala	Tyr	
65				70					75					80		

ctg	aac	ctc	agc	agc	ctg	acc	tct	gag	gac	act	gca	act	tat	ttt	tgt	288
Leu	Asn	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Thr	Ala	Thr	Tyr	Phe	Cys	
85				90										95		

acc	aca	tgg	cgg	ata	tac	cga	gat	agt	tct	ggc	cgc	ccc	ttc	tat	gtt	336
Thr	Thr	Trp	Arg	Ile	Tyr	Arg	Asp	Ser	Ser	Gly	Arg	Pro	Phe	Tyr	Val	
100				105										110		

atg	gat	gcc	tgg	ggt	caa	gga	gct	tca	gtc	act	gtc	tcc	tca		378
Met	Asp	Ala	Trp	Gly	Gln	Gly	Ala	Ser	Val	Thr	Val	Ser	Ser		
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20 25 30

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35 40 45

Gly Arg Ile Asp Pro Glu Asp Asp Ser Thr Lys Tyr Ala Glu Arg Phe
50 55 60

Lys Asp Arg Ala Thr Leu Thr Ala Gln Thr Ser Ser Asn Thr Ala Tyr
65 70 75 80

Leu Asn Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Thr Tyr Phe Cys
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Thr Thr Trp Arg Ile Tyr Arg Asp Ser Ser Gly Arg Pro Phe Tyr Val
100 105 110

Met Asp Ala Trp Gly Gln Gly Ala Ser Val Thr Val Ser Ser
115 120 125

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Gly Tyr Tyr Phe His
1 5

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Gly Tyr Tyr Phe His
1 5

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1 5 10 15

gac 51

<210> 14
<211> 16
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<400> 14
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1 5 10 15

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 1 5 10 15

gcc 51

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 1 5 10 15

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 1 5 10 15

gat caa gcc tcc atc tct tgc aga tct agt cag agc ctt gta cac agt 96
 Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
 20 25 30

aat gga aac acc tat tta cat tgg tac ctg cag aag cca ggc cag tct 144
 Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45

cca aag ctc ctg atc tac aaa gtt tcc aac cga ttt tct ggg gtc cca 192
 Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60

gac agg ttc agt ggc agt gga tca ggt aca gat ttc aca ctc aag atc	240
Asp Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile	
65 70 75 80	
agc aga gtg gag gct gag gat ctg gga gtt tat ttc tgc tct caa agt	288
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser	
85 90 95	
aca cat gtt cct ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata	336
Thr His Val Pro Pro Tyr Thr Phe Gly Gly Thr Lys Leu Glu Ile	
100 105 110	
aaa cgg	342
Lys Arg	

<210> 18	
<211> 114	
<212> PRT	
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Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly	
1 5 10 15	
Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser	
20 25 30	
Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser	
35 40 45	
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro	
50 55 60	
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile	
65 70 75 80	
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser	
85 90 95	
Thr His Val Pro Pro Tyr Thr Phe Gly Gly Thr Lys Leu Glu Ile	
100 105 110	
Lys Arg	

<210> 19	
<211> 48	

<212> DNA
<213> *Mus musculus*

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<222> (1)...(48)
<223> VK13G9 Light Chain CDR I

<400> 19 48
aga tct agt cag agc ctt gta cac agt aat gga aac acc tat tta cat
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1 5 10 15

<210> 20
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1 5 10 15

<210> 21
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<222> (1)...(21)
<223> VK13G9 Light chain CDR II

<400> 21 21
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Lys Val Ser Asn Arg Phe Ser
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<210> 22
<211> 7
<212> PRT

<213> Mus musculus

<400> 22

Lys Val Ser Asn Arg Phe Ser
1 5

<210> 23

<211> 30

<212> DNA

<213> Mus musculus

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<221> CDS

<222> (1)...(30)

<223> CK13G9 Light chain CDR III

<400> 23

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Ser Gln Ser Thr His Val Pro Pro Tyr Thr
1 5 10

30

<210> 24

<211> 10

<212> PRT

<213> Mus musculus

<400> 24

Ser Gln Ser Thr His Val Pro Pro Tyr Thr
1 5 10

<210> 25

<211> 369

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)...(369)

<223> Heavy chain V region

<400> 25

caa gtt act ctt aag gag tct ggc cct ggg ata ttg aag ccc tca cag	1	5	10	15	48
Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Lys Pro Ser Gln					
acc ctc agt ctg act tgt tct ttc tct ggg ttt tct ctg agc act tct	20	25	30		96
Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Ser					
ggt atg ggt att gcc tgg gtt cgt cag cct tca ggg aag ggt ctg gag	35	40	45		144
Gly Met Gly Ile Ala Trp Val Arg Gln Pro Ser Gly Lys Gly Leu Glu					
tgg ctg gca gac att tgg tgg gat gat aat aag tat tat aat cca tcc	50	55	60		192
Trp Leu Ala Asp Ile Trp Trp Asp Asp Asn Lys Tyr Tyr Asn Pro Ser					
ctg gag agc cag ctc aca atc tcc aag gat acc tcc aga aac cag gta	65	70	75	80	240
Leu Glu Ser Gln Leu Thr Ile Ser Lys Asp Thr Ser Arg Asn Gln Val					
tcc ctc acg atc acc agt gtg gac act gca gat tct gcc act tat tac	85	90	95		288
Phe Leu Thr Ile Thr Ser Val Asp Thr Ala Asp Ser Ala Thr Tyr Tyr					
tgt gct cgt cat cat tac gac ggt agt agc ctc ctg cct atg gac tac	100	105	110		336
Cys Ala Arg His His Tyr Asp Gly Ser Ser Leu Leu Pro Met Asp Tyr					
tgg ggt caa gga acc tca gtc acc gtc tcc tca	115	120			369
Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser					
<210> 26					
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<212> PRT					
<213> Mus musculus					
<400> 26					
Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Lys Pro Ser Gln					
1 5 10 15					
Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Ser					
12/21					

20	25	30
Gly Met Gly Ile Ala Trp Val Arg Gln Pro Ser Gly Lys Gly Leu Glu		
35	40	45
Trp Leu Ala Asp Ile Trp Trp Asp Asp Asn Lys Tyr Tyr Asn Pro Ser		
50	55	60
Leu Glu Ser Gln Leu Thr Ile Ser Lys Asp Thr Ser Arg Asn Gln Val		
65	70	75
Phe Leu Thr Ile Thr Ser Val Asp Thr Ala Asp Ser Ala Thr Tyr Tyr		
85	90	95
Cys Ala Arg His His Tyr Asp Gly Ser Ser Leu Leu Pro Met Asp Tyr		
100	105	110
Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser		
115	120	

<210> 27
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<220>
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21

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 <211> 7
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<400> 28
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 1 5

<210> 29
 <211> 48
 <212> DNA

<213> *Mus musculus*

<220>

<221> CDS

<222> (1)...(48)

<223> VH13G9 Heavy chain CDR II

<400> 29

gac att tgg tgg gat gat aat aag tat tat aat cca tcc ctg gag agc
Asp Ile Trp Trp Asp Asp Asn Lys Tyr Tyr Asn Pro Ser Leu Glu Ser
1 5 10 15

48

<210> 30

<211> 16

<212> PRT

<213> *Mus musculus*

<400> 30

Asp Ile Trp Trp Asp Asp Asn Lys Tyr Tyr Asn Pro Ser Leu Glu Ser
1 5 10 15

<210> 31

<211> 39

<212> DNA

<213> *Mus musculus*

<220>

<221> CDS

<222> (1)...(39)

<223> VH13G9 Heavy chain CDR III

<400> 31

cat cat tac gac ggt agt agc ctc ctg cct atg gac tac
His His Tyr Asp Gly Ser Ser Leu Leu Pro Met Asp Tyr
1 5 10

39

<210> 32

<211> 13

<212> PRT

<213> *Mus musculus*

<400> 32

His His Tyr Asp Gly Ser Ser Leu Leu Pro Met Asp Tyr
 1 5 10

<210> 33

<211> 324

<212> DNA

<213> Rattus norvegicus

<220>

<221> CDS

<222> (1)...(324)

<223> Light chain V region

<400> 33

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 Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Leu Gly
 1 5 10 15

gaa act gtc tcc atc gaa tgt cta gca agt gag gac ata tac agt tat 96
 Glu Thr Val Ser Ile Glu Cys Leu Ala Ser Glu Asp Ile Tyr Ser Tyr
 20 25 30

tta gca tgg tat caa cag aag cca ggg aaa tct cct cag ctc ctg atc 144
 Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro Gln Leu Leu Ile
 35 40 45

tat gcc aca aaa agg ttg caa gat ggg gtc cca tca cgg ttc agt ggc 192
 Tyr Ala Thr Lys Arg Leu Gln Asp Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

agt gga tct ggc aca cag tat tct ctc aaa ata agc gac atg caa cct 240
 Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Ser Asp Met Gln Pro
 65 70 75 80

gaa gat gaa ggg gat tat ttc tgt cta cag aat tcc aag ttt ccg gtc 288
 Glu Asp Glu Gly Asp Tyr Phe Cys Leu Gln Asn Ser Lys Phe Pro Val
 85 90 95

acg ttc ggt tct ggg acc aag ctg gag atc aaa cgg 324
 Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg
 100 105

<210> 34
<211> 108
<212> PRT
<213> Rattus norvegicus

<400> 34

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Glu	Thr	Val	Ser	Ile	Glu	Cys	Leu	Ala	Ser	Glu	Asp	Ile	Tyr	Ser	Tyr
				20					25				30		
Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ser	Pro	Gln	Leu	Leu	Ile
				35			40				45				
Tyr	Ala	Thr	Lys	Arg	Leu	Gln	Asp	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
				50			55			60					
Ser	Gly	Ser	Gly	Thr	Gln	Tyr	Ser	Leu	Lys	Ile	Ser	Asp	Met	Gln	Pro
				65			70		75			80			
Glu	Asp	Glu	Gly	Asp	Tyr	Phe	Cys	Leu	Gln	Asn	Ser	Lys	Phe	Pro	Val
				85				90			95				
Thr	Phe	Gly	Ser	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg				
				100			105								

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<223> VK14B7 Light chain CDR I

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1					5					10					

33

<210> 36
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<213> Rattus norvegicus

<400> 36

Leu Ala Ser Glu Asp Ile Tyr Ser Tyr Leu Ala
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<210> 37

<211> 21

<212> DNA

<213> *Rattus norvegicus*

<220>

<221> CDS

<222> (1)...(21)

<223> CK14B7 Light chain CDR II

<400> 37

gcc aca aaa agg ttg caa gat

21

Ala Thr Lys Arg Leu Gln Asp

1 5

<210> 38

<211> 7

<212> PRT

<213> *Rattus norvegicus*

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1 5

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<213> *Rattus norvegicus*

<220>

<221> CDS

<222> (1)...(27)

<223> VK14B7 Light chain CDR III

<400> 39

cta cag aat tcc aag ttt ccg gtc acg

27

Leu Gln Asn Ser Lys Phe Pro Val Thr
 1 5

<210> 40
 <211> 9
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 <213> Rattus norvegicus

<400> 40

Leu Gln Asn Ser Lys Phe Pro Val Thr
 1 5

<210> 41
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 <223> Heavy chain V region

<400> 41

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1			5							10					15	

tct	gtg	aag	ttt	tct	tgc	aaa	gtt	tct	ggc	gat	acc	cct	aca	aca	tac	96
Ser	Val	Lys	Phe	Ser	Cys	Lys	Val	Ser	Gly	Asp	Thr	Pro	Thr	Thr	Tyr	
			20							25					30	

tac	gtg	cac	ttt	gtg	aga	caa	agg	cct	gga	cag	ggt	ctg	gaa	tgg	ata	144
Tyr	Val	His	Phe	Val	Arg	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile	
			35					40						45		

gga	agg	att	gat	cct	gag	gat	act	agt	act	aaa	tat	gct	gag	aag	ttc	192
Gly	Arg	Ile	Asp	Pro	Glu	Asp	Thr	Ser	Thr	Lys	Tyr	Ala	Glu	Lys	Phe	
			50				55							60		

aga	aat	aag	gcg	aca	ttc	act	gca	gat	cca	tcc	tcc	aac	aca	gcc	tac	240
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

Arg Asn Lys Ala Thr Phe Thr Ala Asp Pro Ser Ser Asn Thr Ala Tyr				
65	70	75	80	
ct aac ctc agc agc ctg acc cct gag gac act gca acc tat ttt tgt				288
Leu Asn Leu Ser Ser Leu Thr Pro Glu Asp Thr Ala Thr Tyr Phe Cys				
85	90	95		
acc ata atg cgg tac cat agt acc tat agg gtc tat gtt atg gat ttc				336
Thr Ile Met Arg Tyr His Ser Thr Tyr Arg Val Tyr Val Met Asp Phe				
100	105	110		
tgg ggt caa gga act gca gtc act gtc tcc tc				368
Trp Gly Gln Gly Thr Ala Val Thr Val Ser				
115	120			
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Tyr Val His Phe Val Arg Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile				
35	40	45		
Gly Arg Ile Asp Pro Glu Asp Thr Ser Thr Lys Tyr Ala Glu Lys Phe				
50	55	60		
Arg Asn Lys Ala Thr Phe Thr Ala Asp Pro Ser Ser Asn Thr Ala Tyr				
65	70	75	80	
Leu Asn Leu Ser Ser Leu Thr Pro Glu Asp Thr Ala Thr Tyr Phe Cys				
85	90	95		
Thr Ile Met Arg Tyr His Ser Thr Tyr Arg Val Tyr Val Met Asp Phe				
100	105	110		
Trp Gly Gln Gly Thr Ala Val Thr Val Ser				
115	120			
<210> 43				
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<223> VH14B7 Heavy chain CDR I

<400> 43

aca tac tac gtg cac

15

Thr Tyr Tyr Val His

1 5

<210> 44

<211> 5

<212> PRT

<213> *Rattus norvegicus*

<400> 44

Thr Tyr Tyr Val His

1 5

<210> 45

<211> 51

<212> DNA

<213> *Rattus norvegicus*

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<221> CDS

<222> (1)...(51)

<223> VH14B7 Heavy chain CDR II

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48

1 5 10 15

aat

51

<210> 46

<211> 16

<212> PRT

<213> *Rattus norvegicus*

<400> 46

Arg Ile Asp Pro Glu Asp Thr Ser Thr Lys Tyr Ala Glu Lys Phe Arg
1 5 10 15

<210> 47

<211> 42

<212> DNA

<213> *Rattus norvegicus*

<220>

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<222> (1)...(42)

<223> VH14B7 Heavy chain CDR III

<400> 47

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Met Arg Tyr His Ser Thr Tyr Arg Val Tyr Val Met Asp Phe
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42

<210> 48

<211> 14

<212> PRT

<213> *Rattus norvegicus*

<400> 48

Met Arg Tyr His Ser Thr Tyr Arg Val Tyr Val Met Asp Phe
1 5 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/07349

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07 K 16/18; A61 K 39/395; C12 N 5/12, 15/12
US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/388.1, 388.23; 435/326; 536/23.5; 424/130.1, 139.1, 141.1, 142.1, 145.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

West, US Patent full, STN via medline, caplus, embase, biosis. Search terms, interleukin-18 or IL-18, antibodies, monoclonal, humanized, 2C10, 14B7, 13G9

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	REKVIG et al. Molecular analyses of anti-DNA antibodies induced by polymavirus BK in Balb/c mice. Scandinavian Journal of Immunology. 1995, Vol.41, No.6, pages 593-602. See sequence comparison "A".	14
X	BONILLA et al. V kappa gene usage, idiotype expression and antigen binding among clones expressing the VH _X 24 gene family derived from naive and anti-idiotype immune Balb/c mice. Journal of Immunology. 15 July 1990, Vol.145, No.2, pages 616-622, see sequence comparison "B"	14

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
29 JUNE 2000	04 AUG 2000

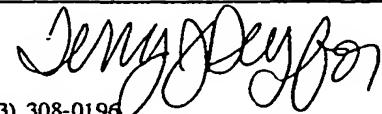
Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
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INTERNATIONAL SEARCH REPORT

International application No. PCT/US00/07349

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JONES et al. Expression of TIMP3 mRNA is elevated in retinas affected by simplex retinitis pigmentosa. FEBS Letters. October 1994, VOL.352, No.2, pages 171-174, see sequence comparison "C"	15
X	EP 0528767 A1 (SANDOZ, LTD.) 24 February 1993 (24.02.93), see page 35, also see sequence comparison "D"	14
A, P	EP 0974600 A2 (KABUSHIKI KAISHA HAYASHIBARA SEIBUTSU KASAKU KENKYUJO) 26 January 2000 (26.01.2000), see entire document.	1-26
A	WO 99/09063 A1 (VEDA RESEARCH AND DEVELOPMENT CO. LTD.) 25 February 1999 (25.02.99), see entire document.	1-26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/07349

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/07349A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

530/388.1, 388.23; 435/326; 536/23.5; 424/130.1, 139.1, 141.1, 142.1, 145.1

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-4, 7-8, 11-26, drawn to a rat monoclonal antibody (Rat 2C10) specific for human IL-18, a hybridoma cell line producing said antibody a nucleic acid encoding said antibody, a pharmaceutical composition comprising said antibody, a method of treatment using said antibody and a method of diagnosis using said antibody.

Group II, claims 1-3, 5, 7, 9, 11-26, drawn to a rat monoclonal antibody (Murine 13G9) specific for human IL-18, a hybridoma cell line producing said antibody a nucleic acid encoding said antibody, a pharmaceutical composition comprising said antibody, a method of treatment using said antibody and a method of diagnosis using said antibody.

Group III, claims 1-3, 6-7, 10-26, drawn to a rat monoclonal antibody (Rat 14B7) specific for human IL-18, a hybridoma cell line producing said antibody a nucleic acid encoding said antibody, a pharmaceutical composition comprising said antibody, a method of treatment using said antibody and a method of diagnosis using said antibody.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Pursuant to 37 C.F.R § 1.475 (d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly, the main invention (Group I) comprises the first-recited product, the Rat 2C10 antibody and the nucleic acid molecule encoding it, a method of using it. Further pursuant to 37 C.F.R § 1.475 (d), the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/07349

methods accordingly defines a separate invention.

Vtk2cl0

1 0 30 15

I D I Q M T Q S P A S L S A S L
 GAC ATT CAA ATG ACC CAG TCT CCA GCT TCC CTG TCT GCA TCT CTG
 CTG TAA GTT TAC TGG GTC AGA GCT CGA AGG GAC AGA CGT AGA CAC

50 70 90 30

CDRI

16 G E T V S I E C L A S E D I Y
 GGA GAA ACT GTC TCC ATC GAA TCT CTG GCA AGT GAG GAC ATA TAC
 CCT CTT TGA CGG AGG TAG CTT ACA GAC UGT TCA CTC CTC TAT ATA

1 10 13 0 45

31 T Y L T W Y Q Q K P G K S P Q
 ACT TAT TTA ACA TCC TAT CAG CAG AAA CCA GGG AAA TCT CCT CAA
 TGA ATA ATT TCT ACC ATA UTC GTC TTT GGT CCC TTT AGA GGA GTT

150 170 60

CDRII

46 I L T Y G A N K L D C V P S
 CTC CTG ATC TAT GGT GCA AAT AAG TTG CAA GAT GCG GTC CCA TCA
 GAG GAC TAC ATA CCA CGT TTA TTC AAC GTT CTA CCC CAG GGT AGT

19 0 210 75

61 R F S C S C S G T O Y S L K I
 CGG TTC ATC GGC ACT CCA TCT GGC ACA CAG TAT TCT CTC AAG ATC
 GCC AAG TCA CGG TCA CCT ACA CCC TGT GTC ATA AGA GAC TTC TAG

2 30 25 0 270 90

76 S G I Q P R D B G D Y F C L Q
 AGC CGC ATA CAA CCT GAA GAT CAA GGG GAT TAT TTC TGT CTA CAC
 TCG CGG TAT GTP GGA CTT CTA CTT CCC CTA ATA AAG ACA GAT CTC

CDRIII

2 90 31 0 105

91 G S K P P L T F G S G T K L E
 GGT TCC AAC TTT CGG CTC ACG TTC GGT TCT GGG ACC AAG CTG GAG
 CCA AGG TTC AAA GGC GAG TGC AAG CCA AGA CCC TGC TTC GAC CTC

106 I K R 108
 ATC AAA CGG
 TAG TTT GCG

FIGURE 1

1/6

VH2C10

1 10 30 15
 1 E V Q L Q Q S G A E L V R P C
 GAG ATC CAG CTA CAG CAG TCT GGG GCT GAG CTT CTC AGA CCT GGG
 CTC CAG GTC GAT GTC AGA CCC CGA CTC CAA CAC TCT GGA CGC

16 50 70 90 30
 T S V K L E C K V S G E I S T
 ACC TCT GTG AAG TTA TCT TGC AAA CTT TCT GGC GAA ATA ACT ACA
 TGG AGA CAC TTC ATA AGA AGC TTT CAA AGA CGG CTT TAT TCA TGT

CDRI

31 1 10 13 0 45
 G Y Y F H F V R R R P G Q G L
 GGA TAC TAT TTC CAC TTT GTG AGG CGA AGG CCT GGA CAG GGT CTC
 CCT ATC ATA AAC CGC AAA CAC TCC GCT TCC CGA CCT GTC CGA GAC

CDRII

46 150 1 70 60
 E W I G R I D P R D D S T K V
 GAA TGG ATA GGA AGG ATT GAT CCT GAG CAT GAT AGT ACT ATA TAT
 CTT ACC TAT CCT TCC TAA CTA GGA CTC CTA CTA TCA TGA TTT ATA

51 19 0 23 0 75
 R E R F K D K A T L T A Q T S
 GCT GAC AGG TTC AAA GAC AGG GCG ACG CTC ACT CGA CAA ACA TCC
 CGA CTC TCC AAC TTT CGT TCC CGC TGC GAG TGA CCT GTT TGT AGG

76 2 30 25 0 270 90
 S N T A Y L N L S S L T S B D
 TCC AAC ACA GGC TAC CTC AAC CTC AGC AGC CTC ACC TCT GAG GAC
 AGG TTG TGT CGG ATG GAC TTC GAG TCG TCG GAC TGG AGA CTC CTG

91 2 90 31 0 105
 T A T Y F C T T W R I Y R D S
 ACT GCA ACT TAT TTT TCT ACC ACA TGG CGG ATA TAC CGA CAT AGG
 TGA CGT TGA ATA AAA ACA TCG TGT ACC GCC TAT ATG CCT CTA TCA

CDRIII

106 330 3 50 120
 S G R P F Y V M D A W G Q G A
 TCT GGC CGC CGC TTC TAT GTT ATG GAT GGC TGG GGT CGA CGA CCT
 AGA CGG CGG GGG AAG ATA CGA TAC CTC CGC ACU CGA GTT CCT CGA

121 37 0 126
 S V T V S S TCA GTC ACT GTC TCC TCA
 AGT CAG TGA CAG AGG AGT

FIGURE 2

V_k13g9

1 0 30
 1 D V V M T O T P I S L P V S L 15
 GAC GTT GTT AGC ACT CAA ACT CCT CTC TCC CTG CCT GTC AGT CTT
 CTG CAA CAA TAC TGA GTT TGA GGA GAG AGG GAC GCA CAC TCA GAA

CDR I
 50 70 90
 16 G D Q A S I S C R S S D S L V 30
 GGA GAT CAA GCG TCC ATC TCT TCC AGA TCT AGT CAG AGC CTT GTC
 CCT CTA GTT CGT AGG TAG AGA AGG TCT AGA TCA GTC TCG GAA CAT

1 10 13 0
 31 H S N C N T V L H W Y I Q X P 45
 CAC AGT ATT CGA AAC ATC TAT TTA CAT TGG TAC CTG CAC AAC CGA
 GTG TCA TTA CCT TTC TCC ATA ATT GTC ACC ATG GAC CTC TTC GGT

CDR II
 150 170
 46 C Q S P K L D I Y K V S N R P 60
 CGC CAC TCT CGA AAG CTC CTG ATC TAC AAA GTT TCC AAC CGA TTT
 CCG CTC AGA GGT TTC CAG GAC TAG ATG TTT CGA AGG TTC CCT AAA

19 0 210
 61 S G V P D R P S G S G S G T D 75
 TCT GGG GTC CGA GAC AGC TTC ACT GGC AGT GGA TCA GGT ACA GAT
 CGA CGC CGT CTG TCC AAC TCA CGG TCA CCT AGT CCA TGT CTA

2 30 25 0 270
 76 P T L K I S R V R B E D L G V 90
 TTC ACA CTC AAG ATC AGC AGA CTG CAC CCT GAG GAT CTG GGA GTT
 AAG TGT GAG TTC TAG TCG TCT CAC CTC CGA CTC CTA GAC CCT CAA

CDR III
 2 90 31 0
 91 V F C S Q S T H V F P Y T P G 105
 TAT TTC TCC TCT CAA ACT ACA CAT GTT CCT CCG TAC AGG TTC GGA
 ATA AAC AGG AGA GTT TCA TGT GTC CAA GGA GCG ATG TGC AAG CCT

330
 106 G G T K L E I K R 114
 GGG GGG ACC AAG CTG GAA ATA AAA CGG
 CGC CGC TGG TTC GAC CCT TAT TTT GCC

FIGURE 3

3/6

VII(B9)

1 0 30 15

1 Q V T E K R S G P G I L K P S
CAA GTT ACT CTT AAG GAG TCT GGC CCT GGG ATA TCG AAG CCC TCA
GTT CAA TGA GAA TTC CTC AGA CCC GCA CCC TAT AAC TTC GGG ACT

50 70 90 30

16 Q T L S L T C S F S G P S T S
CAG ACC CTC AGT CTG ACT TGT TCT TTC TCT GGG TTT TCT CTG AGC
GTC TGG GAG TCA GAC TGA ACA ACA AAG AGA CCC AAA AGA GAC TCG

1 10 13 0 45

31 T S G M G T A W V R Q P S G K
ACT TCT GGT ATG GGT ATT CGC TGG GTT CGT CAG CCT TCA GGG AAG
TGA AGA CCA TAC CCA TAA CGC ACC CAA GCA GTC GGA AGT CCC TTC

150 170 60

46 G L E W L A D I W W D D N K Y
GGT CTG GAG TGG CTC GCA CAC ATT TGG TGG GAT CAT AAT AAG TAT
CCA GAC CTC ACC GAC CCT CTC TAA ACC ACC CTA CTA TTA TTC ATG

19 0 210 75

61 Y N P S L E S Q L T I S K D T
TAT AAT CCA TCC CTG GAG AGC CAC CTC ACA ATC TCC AAG CAT ACC
ATA TTA GGT AGG GAC CTC TGG GTC QAG TGT TAG AGG TTC CTA TGG

2 30 25 0 270 90

76 S R N Q V F L T T T S V D T A
TCC AGA AAC CAG GTA TTC CTC AGC ATC ACC AGT GTG GAC ACT GCA
AGG TCT TTG GTC CAT AAG GAG TGC TAC TGG TCA CAC CTG TGA CCT

2 90 31 0 105

91 D S A T Y Y C A R H H Y D G S
GAT TCT GCC ACT TAT TAC TGT GCT CCT CAT CAT TAC GAC GGT ACT
CTA AGA CGG TGA ATA ATG ACA CGA GCA GTC GAA ATG CTG CCA TCG

3 30 3 50 120

106 S L L P M D Y W C Q G T S V T
AGC CTC CTG CCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC ACC
TUG GAG GAC GGA TAC CTG ATG ACC CCA GTT CCT TGG AGT GAC TGG

121 V S S 723

GTC TCC TCA
CAG AGG ACT

FIGURE 4

VK14B7

1 10 30 15

1 D I Q M T Q S P A S L S A S S I
GAT ATT CAA ATG ACG CAG TCT CCA CCT TCC CTC TCT GCA TCT CTC
CTA TAA GTT TAC TUC CTC AGA GGT CGA AGG GAC AGA CGT AGA GAC

16 50 70 90 30

16 G E T V S I E C 1. A S E D T Y
GGA GAA ACT GTC TCC ATC GAA TGT CTA GCA AGT GAG GAC ATA TAC
GCT CTT TGA CAG AGG TAG CTT AGA CAT CGT TCA CTC CTC TAT ATG

31 110 130 45

31 S Y L A W Y O Q K P G K S P Q
AGT TAT TTA GCA TGG TAT CAA GAC AAG CUA GGG AAA TCT CCT GAG
TCA ATA AAT CGT ACC ATA GTT GTC TTC GGT CCC TTT AGA GCA GTC

46 150 170 60

46 L L I Y A T K R L Q D G V P S
CTC CCG ATC TAT GCC ACA AAA AGC TTC GAA GAT GGG GTC CCA TCA
GAG GAC TAG ATA CGG TGT TTT TCC AAC GTT CTA CCC CAG GGT AGT

61 190 210 75

61 R F S G S G S G T Q Y S L K I
CGG TTC AGT GGU AGT GGA TCT GGC ACA CAG TAT TCT CTC AAA ATA
CCC AAG TAA CGG TCA CCT AGA CCC TGT CTC ATA AGA GAG TTT TAT

76 230 250 270 90

76 S D M Q P E D E G D Y F C L D
ACC GAC ATG CAA CCT GAA GAT GAA GGG GAT TAT TGT TGT CTA CAG
TCC CTG TAC GTT GGA CTT CTA CTT CCC CTA ATA AAG ACA GAT GTC

91 CDR III 290 310 105

91 N S K F P V T F G S G T K I B
AAT TCC AAG TTT CCG GTC AGG TTC GGT TGT GGG ACC AAG CTC GAG
TTA AGG TTC AAA GGC CAG TGC AAG CUA AGA CCC TGG TTC GAC CTC

106 I K R 108
ATC AAA CGC
TAC TTT CCC

FIGURE 5

5/6

VIII4B7

1 8 V Q L O O S G A E L V R P G 15
 GAG GTT CAG CTT CAG CAG TCT CCC CCT GAG CTT GTG AGA CCT CGG
 CTC CAA CTC CAA CTC CTC AGA CCC CGA CTC GAA CAC TCT GGA CCC

16 50 70 90 30
 T S V K F S C R V S G D T P T
 ACC TCT GTG AAG TTT TCT TGC AAA GTT TCT CCC GAT ACC CCT ACA
 TGG AGA CAC TTC AAA AGA ACG TTT CAA AGA CGG CTA TGG GGA TCT

31 CDR I 110 130 45
 T Y Y V D F V R Q R P G Q G L
 ACA TAC TAC GTG CAC TTT GTG ACA CAA AGG CCT GGA CGG GGT CTG
 TGT ATG ATG CAC GTG AAA CAC TCT GGT TCC GGA CCT CTC CGA GAC

46 150 170 CDR II 60
 E W T G R I D F E D T S T K Y
 GAA TGG ATA GGA AGC ATT CAT CCT GAG GAT ACT ACT ACT AAA TAT
 CTT ACC TAT CCT TCC TAA CTA CGA CTC CTA TGA TCA TCA TTT ATA

51 19 D 210 75
 A E K F R N R A T F T A D P S
 GCT GAG AAG TTC AGA AAT AAG CCC ACA TTC ACT GCA GAT CGA TCC
 CGA CTC TTC AAG TCT TTA TTC CGG TGT AAG TGA CGT CTA GGT AGG

76 230 250 270 90
 S N T A Y L N I S S L T P E D
 TCC AAC ACA GCC TAC CTA AAC CTC ACC AGG CTG ACC CCT GAG GAC
 AGG TTG TGT CGG ATG GAT TTG GAG TGG TGG GAC TGG GGA CTC CTG

91 290 310 105
 T A T Y F C T I M R Y H S T Y
 ACT GCA ACC TAT TTT TGT ACC ATA ATG CGG TAC CAT AGT ACC TAT
 TGA CGT TGG ATA AAA ACA TGG TAT TAC CGG ATG GTC TCA TGG ATA

106 CDR III 330 350 120
 R V Y V N D F W G Q G T A V T
 AGG GTC TAT GTT ATG GAT TTC TCC CGT CAA GGA ACT GCA GTC ACT
 TCC CGG ATA CGA TAC CTA AAC ACC CGA GAT CCT TGA CGT CAC TGA

121 V S 122

GTC TCC TC

CGG AGC AC

FIGURE 6
6/6

SEQUENCE LISTING

<110> Holmes, Stephen D.
Ho, Yen Sen
Taylor, Alexander
Abdel-Moguid, Sherin S.

<120> Recombinant IL-18 Antagonists Useful in
Treatment of IL-18 Mediated Disorders

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<140> 60/125,299
<141> 1999-03-19

<160> 48

<170> FastSEQ for Windows Version 3.0

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<213> *Rattus norvegicus*

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<222> (1) ... (324)
<223> Light chain V region

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1 5 10 15

48

gaa act gln: tcc atc gaa tgl ctg gca agt gag gac ata tac act kai.
Glu Thr Val Ser Ile Glu Cys Leu Ala Ser Glu Asp Ile Tyr Thr Tyr
20 25 30

96

144
 t.t.s. a.c.s. t.g.g. t.t.t. c.a.p. c.a.p. a.a.a. t.c.a. t.c.t. c.a.s. c.c.c. t.t.g. s.t.c.
 Leu Thr Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro Gln Leu Leu Ile
 35 40 45

192
 t.t.t. g.t.t. s.c.a. a.a.t. a.a.g. t.t.g. c.a.s. q.a.t. g.g.g. g.t.c. c.c.s. t.c.s. c.g.g. t.t.c. a.g.t. g.g.c.
 Tyr Gly Ala Asn Lys Ile Gln Asp Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

240
 a.g.t. g.g.a. t.c.t. g.g.c. s.c.a. c.a.g. I.t.t. I.t.t. c.c.c. s.e.g. s.t.c. a.g.c. a.t.a. c.s.s. c.c.t.
 Ser Gly Ser Gly Thr Cln Tyr Ser Leu Lys Ile Ser Gly Ile Cln Pro
 65 70 75 80

288
 g.a.a. g.a.t. g.s.s. g.g.g. g.s.t. I.t.t. I.t.t. I.g.t. c.t.c. c.a.g. s.g.t. t.c.c. s.a.g. I.t.t. t.c.g. c.t.c.
 Glu Asp Glu Gly Asp Tyr Phe Cys Leu Gln Gly Ser Lys Phe Pro Leu
 85 90 95

324
 a.c.g. t.c.c. a.g.t. t.c.t. g.g.g. a.c.c. a.s.g. c.t.y. g.h.g. s.t.c. a.s.s. c.s.s.
 Thr Phe Gly Ser Gly Thr Lys Leu Cln Ile Lys Arg
 100 105

<210> 2
 <211> 108
 <212> PRT
 <213> Rattus norvegicus

<400> 2
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 1 5 10 15
 Glu Thr Val Ser Ile Gln Cys Leu Ala Ser Glu Asp Ile Tyr Thr Tyr
 20 25 30
 Leu Thr Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro Gln Leu Leu Ile
 35 40 45
 Tyr Gly Ala Asn Lys Leu Cln Asp Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Ser Gly Ile Gln Pro
 65 70 75 80
 Gln Asp Glu Gly Asp Tyr Phe Cys Leu Gln Gly Ser Lys Phe Pro Leu
 85 90 95

Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg
 100 105

<210> 3
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 <212> DNA
 <213> *Rattus norvegicus*

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 <223> VK2C10 Light Chain CDR I

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 Leu Ala Ser Glu Asp Ile Tyr Thr Tyr Leu Thr
 1 5 10

<210> 4
 <211> 11
 <212> PRT
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<400> 4
 Leu Ala Ser Glu Asp Ile Tyr Thr Tyr Leu Thr
 2 5 10

<210> 5
 <211> 21
 <212> DNA
 <213> *Rattus norvegicus*

<220>
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 <222> {1}...{21}
 <223> VK2C10 Light Chain CDR II

<400> 5
 ggt gca aat gag ttg cca gat 21
 Gly Ala Asn Lys Leu Gln Asp
 1 5

<210> 6
<211> 7
<212> PRT
<213> *Rattus norvegicus*

<400> 6
Gly Ala Asn Lys Leu Gln Asp
1 5

<210> 7
<211> 27
<212> DNA
<213> *Rattus norvegicus*

<220>
<221> CDS
<222> [1]...[27]
<223> VK2C10 Light Chain CDR III

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ctt cag ggt tcc aag ttt ccc ctc aag 27
Leu Gln Gly Ser Lys Phe Pro Leu Thr
1 5

<210> 8
<211> 9
<212> PRT
<213> *Rattus norvegicus*

<400> 8
Leu Gln Gly Ser Lys Phe Pro Leu Thr
1 5

<210> 9
<211> 378
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<213> *Rattus norvegicus*

<220>

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<223> Heavy chain V region

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Glu	Val	Gln	Ileu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Arg	Pro	Gly	Thr		
1				5				10				15					

tct	gtg	aag	tta	tct	tgc	aaa	gtt	tct	ggc	gaa	ata	atg	aaa	gga	tac	96
Ser	Val	Tyr	Ileu	Ser	Cys	Lys	Val	Ser	Gly	Glu	Ile	Ser	Thr	Gly	Tyr	
20				25								30				

tat	ttc	cac	ttt	gtt	ggg	ggg	ggg	cct	ggg	cag	gtt	ctg	ggg	tgg	ata	144
Tyr	Phe	His	Phe	Val	Arg	Arg	Arg	Pro	Gly	Gln	Gly	Ileu	Glu	Trp	Ile	
35				40								45				

ggg	agg	att	gtt	cct	gag	gtt	gtt	atc	192							
Gly	Arg	Ile	Asp	Pro	Gly	Asp	Asp	Ser	Thr	Lys	Tyr	Ala	Glu	Arg	Phe	
50				55								60				

aaa	gac	agg	ggg	acg	ctc	act	gca	caa	aaa	tcc	acc	aca	ggc	tac	240	
Lys	Asp	Arg	Ala	Thr	Ileu	Thr	Ala	Gln	Thr	Ser	Ser	Asn	Thr	Ala	Tyr	
65				70				75				80				

atg	aaa	ctc	agg	acg	ccg	acc	tct	gag	gac	atc	gtt	aaa	act	tat	tct	tgt	288
Leu	Asn	Leu	Ser	Ser	Leu	Thr	Ser	Ser	Glu	Asp	Thr	Ala	Thr	Tyr	Phe	Cys	
85				90								95					

acc	aca	tgg	agg	ata	tac	cgt	gtt	atc	tat	ggc	ccg	ccc	ttc	tat	gtt	336
Thr	Thr	Trp	Arg	Ile	Tyr	Arg	Asp	Ser	Ser	Gly	Arg	Pro	Phe	Tyr	Val	
100				105								110				

atg	gtt	ggc	tgg	gtt	cgt	ggg	gtt	tca	gtc	act	gtc	tcc	tca			378
Met	Asp	Ala	Trp	Gly	Gln	Gly	Ala	Ser	Val	Thr	Val	Ser	Ser			
115				120								125				

<210> 10

<211> 126

<212> PRT

<213> Rattus norvegicus

<400> 10

Glu	Val	Gln	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Arg	Pro	Gly	Thr
1															15
Ser	Val	Lys	Ieu	Ser	Cys	Lys	Val	Ser	Gly	Glu	Thr	Ser	Thr	Gly	Tyr
		20													30
Tyr	Phe	His	Phe	Val	Arg	Arg	Arg	Pro	Gly	Gln	Gly	Ieu	Glu	Trp	Ile
															35
Gly	Arg	Ile	Asp	Pro	Glu	Asp	Asp	Ser	Thr	Lys	Tyr	Ala	Glu	Arg	Phe
															40
Lys	Asp	Arg	Ala	Thr	Leu	Thr	Ala	Gln	Thr	Ser	Ser	Asn	Thr	Ala	Tyr
65					70										80
Leu	Asn	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Thr	Ala	Thr	Tyr	Phe	Cys
															85
Thr	Thr	Trp	Arg	Ile	Tyr	Arg	Asp	Ser	Ser	Gly	Arg	Pro	Phe	Tyr	Val
				100											105
Met	Asp	Ala	Trp	Gly	Gln	Gly	Ala	Ser	Val	Thr	Val	Ser	Ser		
															110
															115
															120
															125

<210> 11

<211> 15

<212> DNA

<213> Rattus norvegicus

<220>

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<222> (1)...(15)

<223> VH2C10 Heavy chain CDR 1

<400> 11

gga	tac	tat	ttc	cac											15
Gly	Tyr	Tyr	Phe	His											
2			5												

<210> 12

<211> 5

<212> PRT

<213> Rattus norvegicus

<400> 12

Gly Tyr Tyr Phe Ile

1 5

<210> 13

<211> 51

<212> DNA

<213> Rattus norvegicus

<220>

<221> CDS

<222> (1)...(51)

<223> VH2C10 Heavy chain CDR II

<400> 13

agg att gat cct gag gag gat agt aca aac ttt gat gag agg ttc aac
Arg Ile Asp Pro Glu Asp Asp Ser Thr Lys Tyr Ala Gln Arg Phe Lys

1 5 10 15

48

gac

51

<210> 14

<211> 16

<212> PRT

<213> Rattus norvegicus

<400> 14

Arg Ile Asp Pro Glu Asp Asp Ser Thr Lys Tyr Ala Gln Arg Phe Lys

1 5 10 15

49

<210> 15

<211> 51

<212> DNA

<213> Rattus norvegicus

<220>

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<222> (1)...(51)

<223> VH2C10 Heavy chain CDR TTT

<400> 15

tgg cgg ata tac cga gat agt tct ggc agc acc ttc tat gtt atg val. 48
 Trp Arg Ile Tyr Arg Asp Ser Ser Gly Arg Pro Phe Tyr Val Met Asp
 1 5 10 15

gcc 51

<210> 16
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 <213> *Baillium norvegicum*

<400> 16

Trp Arg Ile Tyr Arg Asp Ser Ser Gly Arg Pro Phe Tyr Val Met Asp
 1 5 10 15

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 <211> 342
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<220>
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 <223> Light chain V region

<400> 17

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 Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
 1 5 10 15

gag cca gcc lcc atc tct tgc aga tct agt cag agc att gta cac agt 96
 Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
 20 25 30

aat gga aac acc tat tta cat tgg tac ctg cag aag cca ggc cag tct 144
 Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45

cca aag ctc ctg alic lcc aca gtt lcc aac cga ttt tct egg gtc cca 192
 Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60

```

gac agg ttc agt ggc agt gga lca ggt aca met ttc aca ctc gag atc 240
Asp Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65           70           75           80

```

```

arg arg gln gag gat gag val ala glu asp leu cys val tyr phe cys ser gln ser
Ser Arg Val Glu Ala Glu Asp Leu Cys Val Tyr Phe Cys Ser Gln Ser
85          90          95

```

aca cat gtt cct aay lsr: aay ttc gga ggg ggg aca aay ctc gaa ata 336
 Thr His Val Pro Pro Tyr Thr Ile Gly Gly Gly Thr Lys Leu Glu Ile
 100 105 110

aaa cgg
Lys Arg

<210> 18
<211> 114
<212> PRT
<213> Muç muçulm

<400D> 1B

Asp Val Val Met Thr Glu Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 59

Asp Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser
25 50 75

Thr His Val Pro Pro Tyr Thr Phe Gly Gly Thr Lys Ieu Glu Ile

100 100 100

Dyeing

<210> 19
<211> 40

<213> DNA
<213> Mus musculus

<220>
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<222> (1) ... (48)
<223> VK13G9 Light Chain CDR I

<400> 19

aga tct agt cag agc ctt gta cac agt aat gga aac aac ttt ttc cat 48
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1 5 10 15

<210> 20
<211> 16
<212> PRT
<213> Mus musculus

<400> 20

Arg Ser Ser Gln Ser Leu Val His Ser Asn Gly Asn Thr Tyr Leu His
1 5 10 15

<210> 21
<211> 21
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1) ... (21)
<223> VK13G9 Light chain CDR II

<400> 21

aaa gtt tcc aac cgc ttt leu 21
Lys Val Ser Asn Arg Ile Ser
1 5

<210> 22
<211> 7
<212> PRT

<213> *Mus musculus*

<400> 22

Lys Val Ser Asn Arg Phe Ser

1 5

<210> 23

<211> 30

<212> DNA

<213> *Mus musculus*

<220>

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<222> (1)...(30)

<223> CK13G9 Light chain CDR III

<400> 23

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Ser Gln Ser Thr His Val Pro Pro Tyr Thr

1 5 10

30

<210> 24

<211> 10

<212> PRT

<213> *Mus musculus*

<400> 24

Ser Gln Ser Thr His Val Pro Pro Tyr Thr
1 5 10

<210> 25

<211> 369

<212> DNA

<213> *Mus musculus*

<220>

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<222> (1)...(369)

<223> Heavy chain V region

<400> 25

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Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Lys Pro Ser Gln				
1	5	10	15	
acc ctc aat ctc act tct tct tcc tct ggg l.l. act ctc aat aat lcl.	20	25	30	96
Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Ser				
20	25	30		
ggt atg ggt att gcc tgg gtt cgt aat tca ggs aat ggc atg gag	35	40	45	144
Gly Met Gly Ile Ala Trp Val Arg Gln Pro Ser Gly Lys Gly Leu Glu				
35	40	45		
tgg ctg gca gac att tgg tgg gag gat cat aat tat tat aat cca tcc	50	55	60	192
Trp Leu Ala Asp Ile Trp Trp Asp Asp Asn Lys Tyr Tyr Asn Pro Ser				
50	55	60		
ctg gag aac cag ctc aca aat tcc aag gat acc tcc aca aac cag gta	65	70	75	240
Ieu Gln Ser Gln Leu Thr Ile Ser Lys Asp Thr Ser Arg Asn Gln Val				
65	70	75	80	
ttc ctc aac atc acc aat gtg gac act gca gat tct gcc act aat tac	85	90	95	288
Phe Ieu Thr Ile Thr Ser Val Asp Thr Ala Asp Ser Ala Thr Tyr Tyr				
85	90	95		
tgt gat cgt cat cat tac gac gat aat aat aat aat gac tcc	100	105	110	330
Cys Ala Arg His His Tyr Asp Gly Ser Ser Ieu Leu Pro Met Asp Tyr				
100	105	110		
tgg ggt cca yya acc tca gtc acc gtc tcc tca	115	120		369
Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser				
115	120			

<210> 26

<211> 123

<212> PRT

<213> Mus musculus

<400> 26

Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Lys Pro Ser Gln

1 5 10 15

Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Ser

12/21

20	25	30
Gly Met Gly Ile Ala Trp Val Arg Gln Pro Ser Gly Lys Gly Leu Glu		
35	40	45
Trp Leu Ala Asp Ile Trp Trp Asp Asp Asn Lys Tyr Tyr Asn Pro Ser		
50	55	60
Leu Glu Ser Gln Leu Thr Ile Ser Lys Asp Thr Ser Arg Asn Gln Val		
65	70	75
Ile Leu Thr Ile Thr Ser Val Asp Thr Ala Asp Ser Ala Thr Tyr Tyr		
85	90	95
Cys Ala Arg His His Tyr Asp Gly Ser Ser Leu Leu Pro Met Asp Tyr		
100	105	110
Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser		
115	120	

<210> 27
 <211> 21
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (11...21)
 <223> VH1309 Heavy chain CDR 1

<400> 27	
act tct ggt atg ggt att gcc	21
Thr Ser Gly Met Gly Ile Ala	
1	5

<210> 28
 <211> 7
 <212> PRT
 <213> Mus musculus

<400> 28	
Thr Ser Gly Met Gly Ile Ala	
1	5

<210> 29
 <211> 48
 <212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)...(48)

<223> VH13G9 Heavy chain CDR II

<400> 29

gac a.t. tgg tgg gat gat cat aag tat tat aat cca tcc ctg gag aac
 Asp Ile Trp Trp Asp Asp Asn Lys Tyr Tyr Asn Pro Ser Leu Glu Ser
 1 5 10 15

48

<210> 30

<211> 16

<212> PRT

<213> Mus musculus

<400> 30

Asp Ile Trp Trp Asp Asp Asn Lys Tyr Tyr Asn Pro Ser Leu Glu Ser
 1 5 10 15

<210> 31

<211> 39

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)...(39)

<223> VH13G9 Heavy chain CDR III

<400> 31

cat cat tac gac ggt agt agc ctc ctg cct atg gag his his
 His His Tyr Asp Gly Ser Ser Leu Leu Pro Met Asp Tyr
 1 5 10

39

<210> 32

<211> 13

<212> PRT

<213> Mus musculus

<400> 32

Bis His Tyr Asp Gly Ser Ser Leu Leu Pro Met Asp Tyr
 1 5 10

<210> 33

<211> 324

<212> DNA

<213> *Rattus norvegicus*

<220>

<221> CDS

<222> {1}...[324)

<223> Light chain V region

<400> 33

gat aat cca atg aca cag tct cca gat leu cty het gca tct ctg gga 48
 Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Leu Gly
 1 5 10 15

gaa act gtc tcc atc gaa tgt cta gca aat gat gac ata tac aat tat 96
 Glu Thr Val Ser Ile Glu Cys Leu Ala Ser Glu Asp Ile Tyr Ser Tyr
 20 25 30

tta gca tgg tct aca cag aag cca ggg aca tct cct cag ctc ctg atc 144
 Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro Gln Leu Leu Ile
 35 40 45

tat gcc aca aca aag ttg cca gat ggg gtc cca tca cgg lln aat ggc 192
 Tyr Ala Thr Lys Arg Leu Gln Asp Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

aat gga tcc ggc aca cag tat tct ctc aca ata agc gac atg cca cct 240
 Ser Gly Arg Cys Thr Gln Tyr Ser Leu Lys Ile Ser Asp Met Gln Pro
 65 70 75 80

gaa gat gca ggg aat tat ttc tgt cta cag aat leu aag ttt ccc gtc 288
 Glu Asp Glu Gly Asp Tyr Phe Cys Leu Gln Asn Ser Lys Phe Pro Val
 85 90 95

acg ttc ggt tct ggg aca cag ctc gat aca cgg 324
 Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg
 100 105

<210> 34
 <211> 108
 <212> PRT
 <213> *Rattus norvegicus*

<400> 34

Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ser	Ala	Ser	Leu	Gly
1					5					10					15
Clu	Thr	Val	Ser	ile	Glu	Cys	Leu	Ala	Ser	Clx	Asp	Ile	Tyr	Ser	Tyr
				20						25					30
Leu	Ala	Tyr	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ser	Pro	Gln	Leu	Leu	Ile
				35				40					45		
Tyr	Ala	Thr	Lys	Arg	Leu	Gln	Asp	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
				50				55					60		
Ser	Gly	Ser	Gly	Thr	Cln	Tyr	Ser	Leu	Lys	Ile	Ser	Asp	Met	Gln	Pro
				65				70				75			80
Glu	Asp	Glu	Gly	Asp	Tyr	Phe	Cys	Leu	Gln	Asn	Ser	Lys	Phe	Pro	Val
				85				90					95		
Thr	Phe	Gly	Ser	Gly	Thr	Lys	Ile	Glu	Ile	Lys	Arg				
				100				105							

<210> 35
 <211> 33
 <212> DNA
 <213> *Rattus norvegicus*

<220>
 <221> CDS
 <222> (1)..(33)
 <223> VK14B7 light chain CDR I

<400> 35

ctt	gca	tgt	gag	gac	ata	tac	agt	tat	tta	gca					33
Leu	Ala	Ser	Glu	Asp	Ile	Tyr	Ser	Tyr	Leu	Ala					
1				5					10						

<210> 36
 <211> 11
 <212> PRT
 <213> *Rattus norvegicus*

<400> 36
Leu Ala Ser Glu Asp Ile Tyr Ser Tyr Leu Ala
1 5 10

<210> 37
<211> 21
<212> DNA
<213> *Rattus norvegicus*

<220>
<221> CDS
<222> (1)...(21)
<223> CK14B7 Light chain CDR II

<400> 37
gcc uca aac aag ttc caa gat
Ala Thr Lys Arg Leu Gln Asp 21
1 5

<210> 38
<211> 7
<212> PRT
<213> *Rattus norvegicus*

<400> 38
Ala Thr Lys Arg Leu Gln Asp
1 5

<210> 39
<211> 27
<212> DNA
<213> *Rattus norvegicus*

<220>
<221> CDS
<222> (1)...(27)
<223> VK14B7 Light chain CDR III

<400> 39
cta cag aat tcc aag ttt ccc gtc acg 27

Leu Gln Asn Ser Lys Phe Pro Val Thr

1 5

<210> 40
 <211> 9
 <212> PRT
 <213> Rattus norvegicus

<400> 40

Leu Gln Asn Ser Lys Phe Pro Val Thr

1 5

<210> 41
 <211> 368
 <212> DNA
 <213> Rattus norvegicus

<220>
 <221> CDS
 <222> (1) . . . (368)
 <223> Heavy chain V region

<400> 41

gag gtt cag ctt aag cag tct ggg gat gag ctt gtc aca cct ggg aac
 Glu Val Gln Leu Cln Cln Ser Cys Ala Glu Leu Val Arg Pro Gly Thr

1 5 10 15

48

tct gtc aag ttt tct tgc aac gtt tct ggc gat aac cct aca aca tcc
 Ser Val Lys Phe Ser Cys Lys Val Ser Gly Asp Thr Pro Thr Thr Tyr

20 25 30

96

tac gtc cac ttt gtc aca aac agg tct gga cag ggt ctc gaa tgg aca
 Tyr Val His Phe Val Arg Gln Arg Pro Gly Glu Gly Ile Glu Trp Ile

35 40 45

144

gga aag att gat ctc gag mat act agt act aas ttt gct gag aag ttc
 Gly Arg Ile Asp Pro Glu Asp Thr Ser Thr Ile Tyr Ala Glu Lys Phe

50 55 60

192

aga mat aag gca aac ttc act gca gat aca tcc tcc aac aca gca tcc

240

Arg Asn Lys Ala Thr Phe Thr Ala Asp Pro Ser Ser Asn Thr Ala Tyr			
65	70	75	80
Leu Asn Ile Ser Ser Leu Thr Pro Glu Asp Thr Ala Thr Tyr Phe Cys			
85	90	95	
acc ata atg agg tac cat agt acc tat agg gtc tat gtt atg gtc ttc			336
Thr Ile Met Arg Tyr His Ser Thr Tyr Arg Val Tyr Val Met Asp Phe			
100	105	110	
Lys ggt cys gga ser gtc gtc ser gtc tcc Le			
Trp Gly Gln Gly Thr Ala Val Thr Val Ser			368
115	120		
<210> 42			
<211> 122			
<212> PRT			
<213> Rattus norvegicus			
<400> 42			
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Thr			
1	5	10	15
Ser Val Lys Phe Ser Cys Lys Val Ser Gly Asp Thr Pro Thr Thr Tyr			
20	25	30	
Tyr Val His Phe Val Arg Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile			
35	40	45	
Gly Arg Ile Asp Pro Glu Asp Thr Ser Thr Lys Tyr Ala Glu Lys Phe			
50	55	60	
Arg Asn Lys Ala Thr Phe Thr Ala Asp Pro Ser Ser Asn Thr Ala Tyr			
65	70	75	80
Leu Asn Leu Ser Ser Leu Thr Pro Glu Asp Thr Ala Thr Tyr Phe Cys			
85	90	95	
Thr Ile Met Arg Tyr His Ser Thr Tyr Arg Val Tyr Val Met Asp Phe			
100	105	110	
Trp Gly Gln Gly Thr Ala Val Thr Val Ser			
115	120		

<210> 43

<211> 15

<212> DNA

<213> *Rattus norvegicus*

<220>

<221> CDS

<222> {1}...{15}

<223> VH14B7 Heavy chain CDR I

<400> 43

aag tac tac gtq cac

Thr Tyr Tyr Val His

1

5

15

<210> 44

<211> 5

<212> PRT

<213> *Rattus norvegicus*

<400> 44

Thr Tyr Tyr Val His

1

5

<210> 45

<211> 5.1

<212> DNA

<213> *Rattus norvegicus*

<220>

<221> CDS

<222> {1}...{51}

<223> VH14B7 Heavy chain CDR II

<400> 45

agg att gat cct gag gag act aat aat tat gct gag gag ttc aca
Arg Ile Asp Pro Glu Asp Thr Ser Thr Lys Tyr Ala Glu Lys Phe Arg

1

5

10

15

48

aat

51

<210> 46

<211> 16

<212> PRT

<213> Rattus norvegicus

<400> 46

Arg Ile Asp Pro Glu Asp Thr Ser Thr Lys Tyr Ala Glu Lys Phe Arg
1 5 10 15

<210> 47

<211> 42

<212> DNA

<213> Rattus norvegicus

<220>

<221> CDS

<222> {1}...{42}

<223> VK14B7 Heavy chain CDR III

<400> 47

Met Arg Tyr His Ser Thr Tyr Arg Val Val Tyr Val Met Asp Phe 42
Met Arg Tyr His Ser Thr Tyr Arg Val Val Tyr Val Met Asp Phe
1 5 10

<210> 48

<211> 14

<212> PRT

<213> Rattus norvegicus

<400> 48

Met Arg Tyr His Ser Thr Tyr Arg Val Val Tyr Val Met Asp Phe
1 5 10

